

Ownership and the human genome

Unexpectedly rapid progress towards the sequencing and listing of the genes in the human genome has caused a flurry of excitement (and injured pride) in the genome community which is likely to surface in Washington next week.

IN the heroic decades of the United States, when the cry would go up that there was gold in California, or in the Yukon, the still stripling federal government had a simple way of heading off potential conflicts between treasure-hunters: let miners stake a claim to minerals beneath tracts of land marked out by wooden pegs, register their claims and then try to make a fortune while their licences remained in force. The system worked reasonably well; the fisticuffs over disputed claims would have been more frequent without rules of any kind. The latest gold-rush has been stimulated by the suspicion that there are fortunes to be made from the human genome. Through patent laws, some of the same rules appear to apply. But the circumstances are so different that nobody can be sure that trouble will be avoided.

One measure of the difficulties ahead is the informal and private meeting called in Washington for 4 October at which issues of ownership of and access to information about the human genome will be discussed (see page 365). Some of these issues arose just a week ago, over the prospect that lawful patents will restrict access to diagnostic tests eventually based on characterization of the *BRCA1* gene, whose mutations are involved in some familial breast cancer (see *Nature* **371**, 279; 1994). But there is a wider and potentially more corrosive conflict, that between what may be called the public and the private sectors in the custody, communication and exploitation of information about the human genome in general. That is what the next week's meeting is about.

Databases

The issue is typified by the way in which the internationally coordinated databanks storing DNA sequence information are managed. The sequence information now stored is probably a fair reflection of that so far published in the world's journals. Indeed, many researchers submit information about DNA sequences that will never appear as ink on paper. That is proof of the academic community's eagerness to regard the accumulating databases as a communal resource, the whole of which will be more valuable than the sum of its parts. Personal rivalries notwithstanding, there is no better illustration of how the research community can come to regard the understanding of a complex problem as an enterprise to which all manner of people can contribute, if not with flashes of insight then with data that will be more valuable in a database than in a laboratory notebook.

The fly in that ointment is that contributions to the databases have been asymmetrical from the outset. The

academic community has probably contributed everything it could. But there is no reason why the industrial research community should have been similarly forthcoming. No doubt, published sequences have mostly been dutifully deposited in the databanks, but why should a pharmaceutical or other kind of company tell its competitors in which part of the human genome its interests lie, for example by submitting to the databases sequence information not otherwise made public? Or reveal some pattern of mutations in a particular gene from which in due course a drug may be fashioned?

Access

Yet both academic and industrial users have equal access to the databases; such fees as industrial users may pay for access are likely always to be dwarfed by the costs of the software and its maintenance required to make sense of concatenations of four letters from the roman alphabet — **A**, **T**, **C** and **G**. The fear that this asymmetry would become a problem is partly why *Nature* resolved, ten years ago, not to make the submission of sequences to the databanks an absolute and prior condition of publication. It is naive of the research community not to have shared that caution.

By all accounts, part of the occasion for next week's meeting are rumours of the current activities surrounding the pioneering work of Dr J. Craig Venter, now at the Institute for Genomic Research (TIGR) in the United States, but previously an intramural researcher at the US National Institutes of Health (NIH). This is not the first time that Venter has put the cat among the pigeons. When, four years ago, he had developed a technique for identifying the genes expressed in particular tissue cells by short stretches of their DNA, called 'expressed sequence tags' (ESTs), he startled the genome community by publishing some hundreds of them. The numbers of ESTs has since then grown (if rumour is to be believed) into the hundreds of thousands.

Three years ago, NIH caused international consternation by applying for patent protection for Venter's ESTs, stimulating similar protective moves by its counterparts elsewhere. Academies and even governments (France and Britain, for example) protested publicly at the notion of patent protection for naturally occurring human DNA. That issue has now been dropped by the NIH (but not by the company that supports Venter's work, Human Genome Sciences Inc. (HGS)) on the grounds that an EST identifying a gene whose function is not known, while admittedly an artefact in the sense defined by patent legislation, may not be a useful



artefact without functional information. But the present fuss is only peripherally to do with patent rights.

Pride

Part of it has also to do with pride. Many in the genome community were offended that Venter's use of ESTs for the identification of genes, while pointing the way to a rapid listing of the 100,000 or so genes in the human genome, was necessarily an inelegant way of doing so. On the other hand, Venter's method is ideally suited to the needs of the industrial community, which is right in believing that better medicines must come from the identification of genes whose products are involved in metabolic disturbances linked with disease.

One sideshow in next week's fuss is the rumour that Venter has submitted for publication in *Nature* his latest accumulation of data, the outcome of a collaboration between TIGR and HGS, also based in Maryland. There are also rumours that a small number of the ESTs found so far have been withheld because HGS has a commercial interest in their further exploitation. (*Nature* does not comment on rumours of this kind to avoid embarrassment to authors should material be refused or withdrawn.) But even if the rumours were correct in all particulars, the implied objection is that it is improper to publish incomplete datasets seems quite without foundation. Is it seriously intended to imply that no data is better than just some? That sounds like dented *amour propre*.

Furthermore, while others have criticized HGS for the terms on which the company is prepared to collaborate with academic researchers, these are in principle no different from those required by other companies who license out technical information — even if different in scale.

Difficulties

Those working in other fields are well used to analogous difficulties, as when oil-exploration companies select for publication from the information they have won by drilling into the Earth's crust only that which will not inform competitors of potential oil or gas reservoirs. Is genomics that different from geophysics? Even if the rumours about Venter's rumoured publication were correct, it would in any case be prudent of critics to see how their objections are dealt with in whatever may eventually be published. It would also be proper to acknowledge that Venter is under no compulsion to publish anything at all, nor to communicate the sequences of his ESTs to the databases. Nor is it unreasonable that access to Venter's data should be restricted to academic and non-profit researchers. Oil companies do not generally make their exploration data available to their competitors.

The nub of the issue to be haggled over next week extends well beyond that side-issue: what is the status of data gathered with support from public funds and then put into the public domain? And what, when gene-finding begins with the quick and dirty search for ESTs, is to become of the several human genome projects, whose goals are very much grander, but no less important?

Even the first question is no longer simple. Different governments follow different policies on data gathered with

public support, but at some level all share the view that one purpose of academic research is to provide information that will help industry to prosper. The difficulty occasioned by the genome databases is that those who contribute the data are from the international research community, and that the beneficiaries may equally be international.

But the US Congress does not support GenBank at Los Alamos to benefit some company in, say, far-off Switzerland. Nor would it now be feasible (as Dr Wally Gilbert was arguing some years ago) to put the databases on a commercial footing, rewarding researchers for data and selling it on to potential beneficiaries. There is no choice but to regard the databases as an international resource whose public cost is a measure of governments' altruism. The same is true of the genome maps such as that developed in Paris by Génethon and the Centre d'Etudes des Polymorphismes Humaines (CEPH). That is as it should be; understanding how the human genome functions is in the general interest.

Future

The future of the human genome projects is a different kind of question. It would be a great misfortune if those concerned give the impression that they resent being upstaged by Venter's enterprise. There is, of course, a sense in which ESTs have taken the wind out of the sails of the projects as originally described. Especially because ESTs (without further genetic sequencing) are likely to yield quite quickly an understanding of the tissue-specificity of particular genes, much of value will soon be learned of development and differentiation at the molecular level. Rather than seeking to cramp Venter's style, the genome community might usefully seek to emulate his methods. Reports that the Wellcome Trust (Britain's richest research charity) might join in backing such a venture are therefore welcome.

But none of that will dispense with the need eventually to sequence the whole of the human genome (not to mention the genomes of other organisms, plants as well as animals). To understand the role of non-coding DNA in the physical structure of the human genome is one obvious need. To identify whatever clues this material may embody for the course of vertebrate evolution is another. There are the strongest reasons for continuing with the projects now mounted, especially with the complete sequencing of model organisms such as the nematode (*Caenorhabditis elegans*), the plant *Arabidopsis* and yeast. But the best strategy for the human genome would be to begin by fitting Venter's ESTs onto a physical map of the genome while using the data provided to learn more about the intact genes they signpost.

There remains an issue the research community alone cannot decide: the danger that the wish to patent genetic information will be a brake on the availability of important data. In the United States, the test of first publication can sustain claims to priority, but elsewhere the first application secures priority. Restraints on publication would be removed if the rules were internationally uniform — and if the grace period could be longer than the 12 months allowed in the United States. That issue, raised often in the past, is now more urgent. □

'Gene map' plan highlights dispute over public vs private interests

London. Leading geneticists from the United States, Europe and Japan are meeting with representatives of government agencies, private foundations and pharmaceutical companies in Washington next week to discuss launching an international effort to draw up a 'transcript map' of the human genome — the next major step in understanding how the genome functions.

The goal of the meeting is to define a strategy for transferring the growing knowledge of biologically-active DNA sequences on to the physical map of the human genome, a preliminary version of which was published last year (see *Nature* 366, 698; 1994). Such a combination would provide important clues to identifying new human genes.

There is a wide consensus that a transcript map of the whole genome will be invaluable to both molecular biologists and geneticists, as well as to pharmaceutical companies keen to exploit related genes for diagnostic and therapeutic purposes. It is also widely felt that cDNA sequencing has reached a stage at which the creation of such

a map, the estimated cost of which varies from \$10 million to \$75 million, is both timely and feasible.

But there is still a vigorous debate on the best strategy for moving forward. One key question to be addressed at next week's meeting, organized by the Wellcome Trust in London, is whether the construction of a gene map should be primarily a 'public domain' activity, or whether it can be safely left to research groups in the private sector (where over half of all research on the human genome is now carried out).

"Our hope is to develop strategies and identify possible funding sources for establishing an open and searchable DNA database which will be of value to the whole scientific community," says Tom Caskey of the Baylor College of Medicine in Houston, Texas, who will be chairing the meeting.

Craig Venter, president of The Institute for Genomic Research (TIGR) at Rockville in Maryland, whose group has already produced (and expects to publish within the next few months — see page 363) data on

almost 200,000 individual expressed sequence tags (ESTs), feels that transferring such information on to a physical map of the genome is the next important step for molecular genetics.

"I have been arguing for a long time that if our sequences were placed on such a map, it would lead very rapidly to the discovery of the genes responsible for a large number of human diseases," Venter said last week.

Set against this enthusiasm, however, is a sharp debate over whether a transcript map should belong in the private or the public domain. Much of this debate has been triggered by tough demands which Human Genome Sciences Inc (HGS) — the private company which provides the funding for Venter's TIGR, and last year reached a \$125 million agreement with the pharmaceutical company SmithKline Beecham — has been placing on scientists wishing to collaborate with them.

In particular, academic groups interested in working on genes for which HGS has already identified the possible DNA sequences have been asked to sign a "material transfer agreement" (MTA) assigning to HGS first rights to all uses of the gene in question. Although some groups have accepted these terms, others have refused to do so.

HGS has been modifying the contents of its MTAs in the light of outside criticism, and Venter claims he is confident that "a final solution will be reached which will be acceptable to the scientific community". But the experience of individual academic groups (as well as of research managers working for SmithKline's rivals, who have been unwilling to meet HGS's terms for collaboration with industrial partners) has been a prime source of current tensions over the 'ownership' of the proposed transcript map.

Some argue that the speed with which TIGR and other commercially backed operations have succeeded in producing medically useful information about the human genome suggests that fears about the implications of private conduct of the work are unfounded. They point out that all this information will eventually be accessible to academic researchers.

"If we can do things much quicker in the private domain, there is no reason why we should not do it [there]," says Daniel Cohen, director of the Centre des Etudes du Polymorphisme Humain (CEPH) in Paris, and head of the team which produced the first-generation physical genome map last year. (Venter and Cohen are already discussing a collaborative project to map Venter's cDNAs on to this map.)

But others argue equally fervently that the terms for collaboration that have been ►

Merck to back 'public' sequencing

London. The US pharmaceutical giant Merck Sharpe and Dohme is throwing down the gauntlet to its rival, SmithKline Beecham, over what it sees as the latter's attempts to corner the market on the commercial use of DNA sequences from the human genome.

In protest at the terms which it and other companies have been asked to accept in return for access to sequences generated by the Human Genome Sciences Inc. in Rockville, Maryland — in which SmithKline took a major stake last year (see above) — Merck is about to announce plans to fund a separate sequencing operation, all the results of which will be placed unrestrictedly in the public domain through Genbank.

The sequencing will be carried out in the laboratory of Bob Waterston at the Washington University School of Medicine in St Louis. Merck has declined to confirm the costs of the project, which essentially involves duplicating the sequencing work of Craig Venter at HGS's Institute for Genomic Research (TIGR). But its contract with Waterston — which is expected to result in information of 200,000 DNA sequences being made public within a year — is reported to be worth around \$5 million.

Merck justifies its decision to back a 'public domain' database of sequences by claiming that, in requiring scientists to enter into strict agreements on the use of sequence information, HGS and TIGR have failed to meet earlier promises to make

sequencing information publicly available.

HGS's strategy is defended by George Poste, the research director of SmithKline. In an interview last week with *The Wall Street Journal*, he argued that, in addition to the SmithKline's pharmaceutical use of HGS's gene discoveries, "there's absolutely no reason why our genes can't be used as currency" in negotiating "creative alliance frameworks with various partners".

But Merck is challenging this philosophy. Without explicitly naming HGS, it claims that current restrictions on access to sequence libraries are likely to "slow progress" in the field.

"Clearly genomics is going to have an enormous impact on the way in which drug discovery goes forward," says Alan Williamson, vice-president of Merck Research Laboratories. "But we do not think that the human genome should be a proprietary thing; Merck believes strongly that basic biology is not proprietary."

HGS has rejected charges that its licensing policy is slowing up progress in research, or that the terms of its agreements are excessive. "Our data will soon be available to the entire scientific community," says Venter. "If Merck wants access to it, then they should talk to HGS and SmithKline." Merck says that its approach "will ensure fair competition and prevent any organization from cornering the market on the human genome".

D. D.

► demanded by HGS make it essential for the transcript map to be compiled in the public domain, with as few restrictions as possible on who should have access to the data in contains, and on what terms.

"There has been concern voiced by a number of people that a considerable amount of effort is going outside the public domain, and that if [the results] are not going to be easily accessible, then the public domain needs a part in such an important venture," says Michael Morgan of the Wellcome Trust.

Some scientists go further to argue that allowing such a project to be dominated by the private sector will inevitably skew its priorities towards economic and industrial, rather than scientific, goals. "There is a public domain angle to all this, and therefore a need for a slight change in emphasis to make sure that all possible goals are covered," says David Bentley, head of human genetics at the Sanger Centre in Cambridge, Britain's main centre for genome sequencing work which is jointly financed by Wellcome and the Medical Research Council.

But others defend focusing sequencing and mapping activities on areas of the genome of potential value for diagnostic and therapeutic uses. "This is what people are waiting for," says Cohen. "Private companies can operate more quickly and efficiently than academic scientists, and for society, the quicker the work is done the better."

Wellcome officials hope that next week's meeting will provide an opportunity for open discussion between partisans of both points of view. But consensus is likely to be difficult to achieve.

Already there have been tensions caused by the fact that, according to Venter, neither SKB, HGS or TIGR have been invited to attend (even though representatives from several large pharmaceutical companies, such as Glaxo and Merck, will be there, as well as from some small gene-hunting companies, such as Sequana and Incyte).

Supporters of the 'public domain' approach argue that, in order to avoid the excessive influence of vested interests — either from industry or governments — funding for a transcript map should come primarily from non-profit foundations.

In Britain, Wellcome is reported to be contemplating providing several million pounds to the Sanger Centre to work on the transcript map; other funds may be made available in the United States to groups such as researchers at MIT's Whitehead Institute in Boston.

But even the distribution of these funds is likely to prove controversial, as the motives of those favouring a 'public domain' solution are challenged by supporters of the 'private' approach on the grounds that, even if not motivated by financial rewards, the desire for scientific recognition may be playing an equally influential role in determining the position of any one institution in the general debate.

David Dickson

Laboratory explosion prompts warning over gas supplies

London. Researchers from the University of Umeå in Sweden, whose laboratory was wrecked last month by an explosion after a saboteur left the gas to all the Bunsen burners turned on, are urging other scientists to review their security arrangements to ensure that they avoid a similar fate.

The attack took place at the laboratories of the medical biochemistry department at the university, 700 km north of Stockholm. It has led Lars Thelander, head of the department, to question the need for central gas systems in modern laboratories.

One laboratory was completely burnt out in the explosion, which took place on 29 August, and which some staff members think might have been triggered by reports of events at the Rockefeller University in New York (see *Nature* 370, 315; 1994).

A technician found the doors to one of the laboratories on the sixth floor closed, when they were usually left open. She smelt burning, and found a peristaltic pump on fire, which she managed to put out after calling the fire brigade.

Members of the fire brigade smelt gas when they arrived and found that the gas outflow pipe had been lit in another laboratory. As the firemen approached, a bottle of ethanol that had been moved deliberately to be near the flame, melted, caught fire and ignited the gas that had settled near the floor causing an explosion, burning two firemen and injuring the technician.

Reagents and equipment, including centrifuges, microscopes and water baths, worth about Skr500,000 (US\$65,100) were destroyed in the fire that followed. In all it is expected to cost Skr1 million to

rebuild the laboratory.

Tor Ny, a professor of medical biochemistry whose laboratory was destroyed, says he is unaware of any motive for the attack — or who may have been responsible for it.

Gas supplies to about a hundred Bunsen burners had been turned on and the doors closed in laboratories on the top three floors of the building. "The whole building was full of gas. It was just lucky that the whole building didn't go up," says Ny.

Neither Ny nor Thelander believe that the attack was carried out by animal rights campaigners, as their laboratory carries out significantly fewer experiments on animals than the other laboratories in Umeå.

Thelander says that, even though "there are always people who are fired", staff relations at the laboratory are no worse than elsewhere. But several factors suggest that the culprit may be a current or former fellow scientist. One is that the saboteur or saboteurs must have had access to a key to enter the building.

Thelander also believes that the culprit must have been familiar both with the layout of the building and with chemical reagents and laboratory systems, in order to set up the fires and avoid getting hurt themselves.

Thelander says that only one of the laboratories housed in the six-storey building in which the explosion took place regularly uses the central gas system, and that it remained in place primarily because it was needed when the building was originally constructed. "There is now a move to close the central gas [supply] and get rid of the risk," says Thelander. "I'm sure many laboratories could do the same." **Maggie Verrall**

US lifts block on returning waste

Munich. Two ships containing highly radioactive spent fuel from four European research reactors were expected to dock early this week at Sunny Point harbour in South Carolina, after a federal appeals court overturned an injunction against their entry into the United States brought by South Carolina's governor, Carroll Campbell.

The court ruling averted a potential crisis in which the ships, each carrying a cargo of 153 spent fuel rods containing bomb-grade highly enriched uranium (HEU), would be unable either to dock in the United States, or to return home to Europe.

Last June, the US Department of Energy (DoE) agreed to accept 409 spent fuel rods (which it had originally provided), in exchange for an agreement on the part of six research reactors in Europe to convert

to more expensive (but safer) low enriched uranium.

These plans were upset earlier this month when Campbell sought to prevent the ships from unloading their cargo in the United States, arguing that the DoE's plans for the storage of nuclear waste represent an environmental hazard which has not been properly assessed.

Environmental groups joined the DoE and research reactor owners in challenging the injunction because of the ships' vulnerability to terrorist attacks (see *Nature* 371, 192; 1994). The federal court's ruling has deflected further immediate embarrassment. But it does not affect a second decision by the district court in South Carolina to conduct a full hearing in late October over a further 256 fuel rods, due to be shipped from Europe in December.

Alison Abbott

Earmark lobbyists 'paid \$60 million a year'

Washington. US universities spend \$60 million a year paying Washington lobbyists to help them to bypass the scientific peer review process and attract earmarked funding for research facilities and projects, a congressional committee was told last week.

William Ihlandfeldt, vice-president of Northwestern University, Illinois, told a hearing of the House of Representatives Science, Space and Technology Committee, chaired by George Brown (Democrat, California), that his institution pays between \$300,000 and \$400,000 a year on lobbying for funds in Washington, and that "more than fifty, I'd say a couple of hundred" other institutions do the same. David Minge (Democrat, Minnesota) noted that this implied a total spend of \$60 million a year.

The news will not surprise the restaurateurs on whose premises the lobbyists — many of them former congressmen or their staff — ply their trade. But it shocked some members of the committee. Harris Fawell (Republican, Illinois), co-chairman of the informal "pork-busters group" in Congress, described it as a "devilish situation".

M. R. C. Greenwood of the White House Office of Science and Technology Policy told the hearing that growth in earmarking "has the potential of destroying the process" that funds scientific research in the United States. But she declined to promise any action by the administration to stop it, and appeared to pass the buck by describing it as a problem first for Congress, and then for the universities that benefit.

Brown wants the administration to issue an executive order reminding federal agencies and government departments that they are not obliged to obey the report language on budget bills, which contains the earmarks. Greenwood declined to offer this, saying that "we have not been able to find a single solution to the problem".

In a follow-up hearing the next day on earmarks by the Department of Defense, John Silber, the president of Boston University, told the committee that he spent \$400,000 each year on the services of a lobbyist, Gerald Cassidy, who had come to his attention through his work for nearby Tufts University. Silber said he had closed a Washington liaison office costing \$250,000 a year to run, and turned to Cassidy instead.

The total cost of individual universities' lobbying efforts is difficult to estimate. But as neither Boston nor Northwestern are likely to be the biggest spenders, and well over a hundred schools are active in the earmark hunt, the \$60 million figure is probably not far from the truth.

"In effect, you robbed banks because that's where the money is," Brown told Ihlandfeldt and other university witnesses. "The agencies fund it, as you have heard, because they don't want to upset senior

congressmen, but there is no law or reason to it. We are not condemning the process because it produces bad science: we are condemning it because it is unfair."

After vigorously defending the way in which Boston University obtained congressional support for its proposed photonics research centre (see *Nature* 371, 273; 1994) without going through the normal peer-reviewed channels, Silber was rebuked by Martin Hoke (Republican, Ohio). "It is clear that you have decided there is an agenda for Boston University which is of greater importance than the agenda of the United States Congress," he told the Boston president.

Earlier, the hearing heard that the Environmental Protection Agency (EPA) had accepted instructions over the telephone

from congressional staff to place three projects at the University of Arkansas, Louisiana State University and the University of North Dakota respectively. In each case, the projects had not been earmarked in the report language of the EPA's budget bill — and were therefore assumed to be open to competitive application.

To some, this suggests the possible existence of a pot of 'invisible' earmarks of indeterminate size, not included in report language or noted in previous earmark tallies and perhaps, say opponents, testing the boundaries of propriety. John Cannon, assistant administrator of EPA, told the hearing that he was not briefed to explain these cases. But Brown's committee is unlikely to let the matter rest.

Colin Macilwain

Virtual reality faces hardware gap

Washington. Awkward headsets and other hard-to-use equipment threaten to obstruct the progress of virtual reality technologies, according to a report published last week by the US National Academy of Sciences on how the federal government should approach interactive computer systems.

The 500-page report, prepared for the government by the National Research Council (NRC), the research arm of the academy, says that virtual reality is largely driven by computer software specialists who tend to ignore the deficiencies of the hardware.

"The importance of adequate hardware tends to be underplayed by the [virtual reality] community," the academy says. "If the comfort of virtual reality systems (particularly head-mounted displays) cannot be radically improved, the practical usage of these systems will be limited to emergency situations or to very short time periods."

Despite the excessive hype for virtual reality, the report concludes that the technology has genuine potential in a wide range of applications, and is engaging the serious research interest of scientists, social scientists and engineers.

Development of virtual reality systems is at present being driven by pressures from two extremes: at one end, the entertainment industry is pursuing low-cost systems, while the military is building highly sophisticated systems with little regard to cost. But the report identifies four sets of intermediate applications — industrial use, medicine, hazardous operations and training — as the most promising domains for the use of virtual reality in the long run.

It proposes that the government should set up "national research and development teams" for different application areas to overcome "organizational barriers" between different agencies, universities and indus-



Microworld: technology opens new opportunities for biologists.

trial companies. It identifies these as a greater problem than relations between different disciplines, such as engineering, computer science and psychology, which are working together on virtual reality.

The report also suggests that the US government should establish a set of accepted standards, or an independent laboratory for formal evaluation of virtual reality equipment. "In general, technology and equipment are not being adequately evaluated," it says. The report identifies various points on which federal research should focus, but makes no recommendations on the money that needs to be spent.

The National Aeronautics and Space Administration, the National Science Foundation and various defence and intelligence agencies jointly commissioned the report from the NRC in 1992. It was conducted by a panel of engineers and scientists chaired by Nathaniel Durlach of the electrical engineering and computer science department at the Massachusetts Institute of Technology.

C. M.

US laboratories under fire over government controls

Washington. The head of an independent inquiry into the future of the laboratories funded by the US Department of Energy (DoE) has expressed "grave concern" that the department is encouraging tighter supervision of the laboratories by government officials, rather than giving contractors a free hand to manage them.

Robert Galvin, chairman of the electronics company Motorola, claimed during a meeting last week of the task force he heads that, in some DoE documents, "every paragraph predicts greatly increased involvement by the Department of Energy in managing the contractor".

The documents include the report published in February of the Contract Reform Team set up by Hazel O'Leary, the Secretary of Energy, which sets out long lists of new departmental functions. On overtime policy, for example, it recommends that "existing controls [should] be expanded to ensure the most appropriate and judicious use of contractor overtime".

Galvin, who was questioning witnesses about a new contract to operate the Idaho National Engineering Laboratory, said he was unsure whether, even under new contracts, such intense oversight gave contractors the freedom to manage sites effectively.

The laboratories employ 30,000 scientists and engineers, and many more support staff. They are designated as government-owned, contractor-operated facilities, or 'Go-Cos'; but Galvin said that some of the words and actions suggested they are "government-owned, government-operated" facilities.

Galvin later said that his remarks were "not intended to be accusative" but were to help the task force to pursue its inquiries, now close to completion. But their tone was reinforced by Will Happer of Princeton University, head of energy research under the Bush administration, who warned the hearing of a department approaching "the Soviet style of management, with a few people dealing with real problems, underneath a vast management bureaucracy".

Happer said that the Go-Co concept "is being steadily eroded by DoE oversight which goes far beyond the real needs of the taxpayer". In his time at DoE, Happer said, "we increasingly had people meddling in the smallest detail", with reports arriving on his desk "which I never read, and no-one on my staff did either". Happer said he placed the blame not on past or present administrations, but squarely with Congress and its steady output of regulations.

The long-standing tension between scientists in the laboratories and DoE officials has recently been aggravated by the dominant view of the former — especially on the weapons and nuclear energy side — that the current administration is hostile towards their work.

The hottest issue to be tackled by the Galvin task force is the rationalization of the three nuclear weapons laboratories — Los Alamos and Sandia in New Mexico, and Lawrence Livermore in California. One outcome thought to be under consideration is a new mission for Lawrence Livermore as a national environmental laboratory.

Colin Macilwain

Britain to rebuild scientific ties with South Africa

Cape Town. After years of separation, Britain and South Africa have announced plans to renew formal scientific links and provide official backing for closer scientific collaboration. John Major, the British prime minister, and Nelson Mandela, the South African state president, last week signed a declaration of intent to draw up a bilateral agreement on science and technology.

Announcing this move in his address to the South African parliament, Major referred to research in pharmaceuticals, water treatment and food production, and the teaching of science and mathematics in schools, as areas in which British expertise could be of assistance to South Africa.

Major was accompanied on his visit by Sir Michael Atiyah, president of the Royal Society, and Sir William Stewart, his chief scientific adviser, as well as officials from the Office of Science and Technology. In addition to talks with government ministers and other officials, the delegation visited universities and research councils.

Stewart later described the meetings as "very positive". He said that Britain has offered to provide advice on restructuring South Africa's science system to meet its economic and social priorities, and on transferring scientific results to industry and other bodies responsible for its application. Funds for such an initiative were likely to be approved by the British government once a programme was established, Stewart said.

Friedel Sellschop, deputy vice-chancellor (research) at the University of the Witwatersrand, and co-chairman of the ministerial advisory committee for arts, culture, science and technology, has now been invited to coordinate proposals for collaboration in preparation for a future visit by David Hunt, the British minister for science.

Some individual negotiations are already taking place. The South African Foundation for Research Development, for example, is discussing closer collaboration in astronomy with the UK Particle Physics and Astronomy Research Council. New agreements are being proposed on both the South African Astronomical Observatory (SAAO) at Sutherland in the Northern Cape Province, and the Radio-astronomy Observatory at Hartebeeshoek in the Pretoria-Witwatersrand-Vereeniging Province.

Atiyah promised close links between the Royal Society and the new South African Academy of Science, which is due to be established later this year. As South Africa rejoined the Commonwealth in July, South African scientists will once again be eligible for election as fellows of the Royal Society.

Michael Cherry

Gupta censured — but keeps his job

New Delhi. Viswa Jit Gupta, the geologist at the Panjab University in Chandigarh, India who was found guilty earlier this year of faking Himalayan fossils (see *Nature* 369, 698; 1994), has been barred from any administrative post in the university, and will receive no further annual salary increments. But his job remains secure.

The punishment was adopted last week by the senate of the university. This followed a four-hour discussion of the report of an inquiry, headed by retired justice M. S. Gujral, which found Gupta guilty of plagiarism, presenting stolen fossils, falsifying data and claiming discoveries from non-existing localities.

Five of the university's 55 senators called for Gupta's dismissal. But the majority favoured leniency as he "has been punished enough through the mental agony he had undergone".

The verdict draws the curtain on a five-year old controversy which was initiated by an article in *Nature* by John Talent, an Australian geologist critical of Gupta's work (*Nature* 338, 613; 1989). But those who were hoping that Gupta would be dismissed claim that he has got off lightly. Gupta has eight more years before retirement, and continues to hold the post of professor with the authority to supervise graduate students.

Gupta continues to dismiss the entire episode as "a conspiracy by foreigners". In a recent interview with the magazine *The Week*, he says that he still stands by all his work since 1963. "I have no intention of reviewing the papers just because of this controversy," Gupta says. "In science every one has a right to publish his own opinion based on observation."

K. S. Jayaraman

Ethics treaty to target genome implications

Paris. Prospects for reaching a consensus on an international agreement on bioethics came a step closer last week with the second meeting of the United Nations Educational Scientific and Cultural Organisation's (UNESCO's) International Bioethics Committee (IBC) in Paris.

The organization is expected to approve a declaration on 'the protection of the human genome' in 1998, and to follow it up with a treaty that would be binding on its signatories. The declaration would be modelled on the 1948 Universal Declaration of Human Rights, and the committee hopes to give it a long shelf-life by avoiding "dogma" and remaining "open to scientific progress", says its chairwoman, Noëlle Lenoir.

The unexpected degree of consensus stems largely from the committee's decision to concentrate on issues raised by genome research, rather than more controversial issues such as patents or therapeutic abortion. The principles of human dignity, of free and informed consent, and of the confidentiality of genetic data that can be traced back to an individual, make up the proposed declaration's backbone.

Indeed, 'genetic reductionism' — the tendency to "stigmatize, ostracize or eliminate" individuals or groups not meeting a genetic norm — emerged as the biggest threat from genome research during the three-day debate. "We must make sure that the folly of genetic purity does not replace the folly of racial purity", says Lenoir.

According to the International League of Societies for Mentally Handicapped Persons, for example, "invisible" social, legal and financial pressures are already forcing women to abort disabled fetuses. Genome research, it warned the committee, could "geneticize social policy", and erode public support for disability healthcare.

The state's duty to support those genetically at risk from illness or handicap is emphasized in the proposed UNESCO declaration, which adds that individuals cannot be "reduced to their genetic characteristics" or be discriminated against because of them.

A second message of the meeting was that too much attention has been paid to the

negative aspects of genetics research. "The heat has to be taken out of the bioethics debate," says Lenoir. Bioethics itself has to switch from a "defensive approach" to a "balanced outlook that considers risks and benefits", she says.

Indeed, some favourite bioethical arguments took a hammering at the meeting. Excessive discussion of "notional" gene therapy has been a "mistake", arousing popular fears and misjudgements of present issues "for no good end", concludes a preliminary report on gene therapy drawn up by one working group.

Many bioethicists, it argues, have mistakenly assumed that somatic gene therapy is about treating genetic diseases. But its main use will be to treat common diseases: cells might be genetically engineered to provide protection against the side-effects of treatments such as cancer therapy, or against HIV.

The real risk of somatic gene therapy, it asserts, is that medical enthusiasm may override human rights during its experimental phase. But once the technique becomes commonplace, it will be no more dangerous than any other therapy, and should be regulated in the same way.

The report also argues that somatic gene therapy for 'enhancements' should not be dismissed as unethical. Medical progress leads to the redefinition of many conditions — such as memory loss with age — as 'diseases', it says, while somatic cell enhancement is no different from cosmetic surgery.

Similarly, the working group's report says that although germline gene therapy should be banned for enhancement purposes, it should not be banned for therapeutic uses. "If it is morally acceptable to cure a condition by somatic gene therapy, then why not to eliminate it by the germline?" asks the report.



Lenoir: 'we must avoid the folly of genetic purity'.

It also says that debates about germline gene therapy, and 'directed evolution', amount to much ado about nothing. The only application envisaged, namely to spare descendants a serious disease, could ultimately be achieved more cheaply by sorting sperm, or by selecting embryos (as is already done in several countries).

Developing expensive germline therapy for individuals who disapprove of abortion or selective implantation, or who are unable to produce disease-free embryos, is not a public policy option, says the report, primarily because of the expense. But banning the technique is premature and unnecessary, as a valid need might arise.

The report of another working group presented to the committee claims that the focus of genetic screening is also shifting from genetic to common diseases. By the end of

the decade, genetic factors will be found for around one-quarter of cancers and heart diseases, says David Shapiro, the group's rapporteur and executive secretary of the UK's Nuffield Council of Bioethics.

One implication, says Shapiro, is that the proposed declaration should consider ignoring the contentious issue of therapeutic abortions for serious genetic disorders, as these will in future represent an increasingly small proportion of screenings.

At the same time, genetic testing will increasingly yield information about an individual's predisposition to multigenic or multifactorial conditions. Screening should therefore be restricted to serious disorders, in particular those that strike before adulthood, says the report, while screening for late-onset diseases should be done with "great care". The greater the availability of treatment, the better the case for screening.

Screening should also be voluntary, says Shapiro, and genetic information should in general remain confidential. Abortion should be banned, he says, for cosmetic reasons, or to avoid normal traits (such as less than average size) or a predisposition to treatable diseases.

Despite a growing consensus about the content of the proposed declaration, there was wide concern among those attending the Paris meeting that a lack of precision in its wording could reduce its effectiveness.

But Lenoir, who is a lawyer, argues that such "constructive ambiguity" is needed to ensure the flexibility that will get consensus. The value of international law, she says, is that it provides a reference for national legislators, and a last resort to which victims can appeal.

Declan Butler

French ministers face poisoning charges

Paris. Formal charges of "collusion in poisoning" are expected to be made this week against Laurent Fabius, the former Socialist prime minister, and two of his cabinet colleagues — Edmond Hervé and Georgina Dufoix — over their role in France's HIV-contaminated blood scandal in the mid-1980s. The charge carries a maximum sentence of 30 years in prison.

Many scientists and physicians remain convinced that the two physicians already convicted in a magistrate's court on the

misdeemeanour of distributing factor VIII contaminated with HIV in 1985, did not receive fair trials. But both physicians, and one other, now also face the charge of poisoning (see *Nature* 370, 404; 1994).

Some feel that this criminal trial, along with that of the three politicians, may finally provide an unequivocal explanation of the events of 1985. Fabius has said that he wishes that "the judiciary will accomplish its mission so that the truth be clearly established".

D. B.

NIH gets the green light on embryo research

Washington. Amidst a flurry of last-minute opposition, an *ad hoc* panel of the National Institutes of Health (NIH) this week released a report that may pave the way for an end to a 13-year *de facto* ban on the federal funding of embryo research.

On Monday, a federal judge denied a request for an injunction to stop the work of the panel. The suit was brought by the International Foundation for Genetics Research, a group in Pittsburgh that funds genetics research and believes that life begins at the moment of conception.

The suit claimed that the NIH had not complied with the law in choosing the members of the panel, and that some panel members had a conflict of interest because they stood to gain research funds for embryo research. But the judge rejected the groups' efforts to prevent the panel's report being presented to Harold Varmus, director of NIH, to reject the panel's findings.

Varmus appointed the panel after Congress had overturned a regulation requiring proposals for publicly funded embryo research to be reviewed by an Ethics Advisory Board (the Reagan and Bush administrations effectively stopped all such research by not appointing anyone to this board). The panel was asked to determine what research outside the womb is acceptable on human embryos created during *in vitro* fertilization, and on parthenotes — eggs stimulated to develop in the absence of sperm.

Earlier this year, the new panel agreed that federal funds should in principle be spent on embryo research. It also agreed that a distinction should be made between embryos intended for transfer and those not, with the greater need for restrictions on

research on the former.

In its final report, due to be published this week, the panel says that, as in Britain and Canada, research on embryos should be restricted to the first 14 days after fertilization. This is the stage at which the primitive streak appears *in vivo*, although because development is slower *in vitro* than *in vivo*, the panel accepts that some research could be done beyond 14 days if it is intended to identify reliably when the primitive streak appears.

The panel recommends that all embryo research should meet certain conditions. For example, it should be clear that the research goals cannot be met using either animals or gametes. Furthermore the experiment should be performed in the shortest possible time, and there should be no purchase of gametes or embryos.



pressure from critics

Much of the panel's debate has focused on the sources of gametes and embryos, such as spares from infertility treatment, women undergoing pelvic surgery, healthy volunteers, women and girls who have died, and aborted fetuses.

The panel says that healthy women, even if volunteers, are not acceptable as donors because of the risks associated with harvesting the eggs. Those undergoing surgery or infertility treatment are acceptable, provided that there is no sale involved and informed

consent has been received.

Similarly, research can be carried out on an embryo from a woman or girl who has died, providing it is not intended for transfer to a uterus. But, aware of the strong emotions surrounding abortion, the panel says that it would be "unwise public policy" to allow aborted fetuses to be used as sources, irrespective of whether transfer is intended.

Judging by the letter from Congress, written by anti-abortionist Robert Dornan (Republican, California) and signed by 26 fellow congressmen, equally strong emotions are likely to surround the panel's view that research on parthenotes is acceptable providing that the activated egg is not transferred to a woman.

The panel says research on parthenotes would provide information about eggs to activate and sustain their early development, and points out that parthenotes fail to develop because they lack essential genes contributed by sperm. But Dornan says that scientists might grow stem cell lines from parthenotes to develop tissue for transplant to adults. "This creation of new life for the purpose of vivisection will be abhorrent to many Americans," the letter says.

While the list of research that it proposes should be forbidden took the panel little time to draw up, decisions on which techniques should be subject to further review were more difficult. Included in this category, for example, was research carried out on an embryo a few days after the appearance of the primitive streak, something that could aid the understanding of the large number of human diseases that arise at this time.

More contentious was the issue of whether to allow the nucleus from one woman's egg to be transplanted to another's. The panel eventually agreed to recommend that this should not be allowed if the procedure was intended either to duplicate a genome or to increase the number of embryos with the same genotype. But because the mother's mitochondrial DNA causes some inherited diseases, the panel also concluded that further review is needed of whether to allow research intended to avoid such diseases.

It is now up to Varmus to decide which of the panel's recommendations to accept. NIH expects the full guidelines to be completed by next summer and that, although they will apply only to publicly funded research, they should also serve as a model for privately funded work.

But Varmus's task will not be easy. NIH has received 18,000 letters, most expressing opposition to all research on human embryos, and individual panel members have received many hundreds more. The NIH has been sufficiently concerned to provide security guards for the protection of panel members during public meetings on the topic.

Helen Gavaghan

LHC caught in French budget crunch

Paris. The new French academic year kicked off last week with François Fillon, the French minister of research and higher education, trying to find a way out of the impasse over funding prospects for the Large Hadron Collider (LHC) being planned at the European Laboratory for Particle Physics (CERN).

Germany and the United Kingdom are demanding that France and Switzerland, as the host nations, should pay an extra 10 per cent of its costs. But France and Switzerland are not prepared to go higher than the 4.3 per cent they recently offered (see *Nature* 371, 187; 1994). "Ten per cent is out of the question," says Fillon, who says that he has written to his British and German counterparts to request a mini-summit to negotiate a settlement.

France's new austerity budget, announced in Paris last week, leaves Fillon with little room for manoeuvre. The government has virtually frozen public spending, scheduled

to increase by just 1.9 per cent, in a bid to reduce its spiralling budget deficit from FF301.4 billion (4.1 per cent of gross national product, GNP) to FF274.6 billion by next year.

Spending on research does somewhat better than many other areas, with an increase of 3.5 per cent to FF52.573 billion, sufficient to keep spending constant as a proportion of GNP at 2.41 per cent.

Within the budget there are few surprises. The worst news for scientists came in a separate announcement from Fillon that the freeze of 8 per cent of the research budget (excluding salaries) the government introduced in June is unlikely to be lifted, as had earlier been indicated.

One change this year is that the budget of the French space agency CNES is to be kept virtually constant, having enjoyed almost double-figure growth through the 1980s.

Declan Butler

Costs row dominates patent office debate

Munich. Conflicting views over the future of the European Patent Office (EPO) in Munich have been brought to a head by a dispute over the appointment of a new president to succeed Paul Braendli when his second five-year term of office runs out next year.

Concerned at the heavy costs of financing the office, some of its 17 member states are suggesting that much of its work could be decentralized to national patent offices. Any such move, however, is being resisted by the staff of the EPO, and some former officials, who claim that in many cases excessive costs can be blamed on the policies of the member states themselves — for example, in demanding unnecessary translation of patent documents — and that decentralization would weaken the EPO's role in boosting European industry.

The EPO accepts patent applications from any country. Applicants then designate the EPO's member states in which they want patent protection, each country costing DM390 (US\$250). Total European patent fees (exclusive of patent agent charges) for seven designated countries average DM11,000.

Arguments for decentralization are closely linked to arguments for reducing the high costs of patenting through the EPO. Erich Häussler, for example, president of the German patent office which is also based in Munich, claims that some national offices could issue European patents and save the cost of running a big central organization.

"This is theoretically feasible, in particular because we can handle different languages such as English," says Häussler, who also wants a "work-sharing cooperation" with the EPO to keep fees down. This would involve the German patent office taking on some search and examination functions for the EPO.

The United Kingdom has floated the idea of five-year exchanges of patent examiners between national patent offices and the EPO, although it is not clear that this would be cheaper. Both Britain and Germany have expressed concern about the high running costs of the large riverside offices and well-paid staff.

But Johannes van Benthem, a former president and one of the founders of the EPO, claims that any split will threaten the principles on which the office was founded, namely the harmonization of European economic policy, and improvement of Europe's ability to compete with the United States and Japan.

He is particularly concerned that patent searchers and examiners from different countries should continue to work closely together in order to avoid national differences and meet common standards in deciding on

patent applications.

Van Benthem believes that the national policies of EPO's member states, rather than the centralized administration, have been responsible for the high cost of European patents. Contracting states insist on both the translation of every patent into their national languages, and on holding back a high proportion of the annual renewal fees that they charge for European patents.

According to the European Patent Convention (EPC), European patents can be submitted and processed in any one of the three official EPO languages, English, Ger-



The European Patent Office: too expensive?

man and French. When granted, the patent specification is published in the chosen official language; only the summarized claims of patents are translated into the other two.

A clause in the convention, however, allows all member states to ask for translations of patents registered in their country into the national language. The result, according to van Benthem, is that patentees spend about DM1 billion a year, an amount equal to the EPO's total operating costs, on translations that are rarely consulted and have no legal validity (this is the preserve of originals only). "It has moved the political and cultural problem of language into the economic arena where it does not belong," he says.

Annual renewal fees are also a source of lost income to the EPO. These are charged on all patents to keep them active; they are set independently of the EPO by the national

patent offices, and paid directly to the national offices by the patentee. The charge is intended to cover administrative costs of keeping the patent alive, but national offices return only half of the money they receive to the EPO. "This is a tax on innovation for which national patent offices do virtually nothing," says Paul Luckett, deputy chairman of the Munich branch of the staff union.

The EPO acknowledges that filing for European patents is relatively expensive, and is studying ways in which costs could in particular be reduced for small businesses. According to EPO officials, this study is looking at the two major points raised by van Benthem, accepting that these are the main sources of high costs.

But whatever the study reveals, officials of the staff union believe that the choice of the next EPO president will have a strong influence on the outcome of debate over decentralization, as some delegations, in particular the Germans, have the issue high on their agenda.

Union officials fear that an apparent rush to complete the vote before the German general elections in the middle of October could damage the EPO by reducing the opportunity for new candidates to come forward beyond those already nominated if Braendli withdraws, having failed to win sufficient support for his nomination. In particular, they are concerned that the election of the German candidate, Ingo Kober, would bring the dispute with the German patent office in-house as a result of his political connections with the national body.

Kober is a member of the small but influential Free Democratic Party (FDP) which currently occupies key cabinet positions in Bonn, but may lose its place in the government after the general elections if it gains fewer than five per cent of the votes cast. He is currently the permanent secretary in the Ministry of Justice — whose minister, Sabine Leutheusser-Schnarrenberger, is also an FDP member — and worked for a time in the personnel department of the German patent office.

Alison Abbott

Germany holds back on university support

Munich. Germany's 16 *Länder* last week finally accepted the federal government's offer to pay DM1.8 billion over the next four years towards building and repair costs of universities — a figure that is considerably below what they had been hoping for.

The Wissenschaftsrat, Germany's influential science advisory council, had recommended that the government pay DM2.3 billion towards building costs, which would have been equalled by the *Länder* under the scheme of cost-sharing for universities.

The council said this money was needed to maintain academic standards and meet new political demands on higher education, such as the expansion of Fachhochschulen (technical colleges). The federal government, however, citing general budgetary constraints, claims the figure is too high. After months of negotiation, the *Länder* have finally backed down. Karl-Heinz Hoffmann, head of the Wissenschaftsrat, says the compromise will have "serious consequences" for German universities. **A. A.**

US energy policy folly

SIR — During the 1950s and 1960s, published studies forecast that a permanent decline in US oil production would start around 1970 (refs 1,2). Industry and government regarded these warnings as crying wolf; the accepted view was that the United States had all the oil it would need for the foreseeable future^{3,4}.

When the predicted decline in US oil production arrived on schedule, it was ignored; the United States simply imported more oil, doing nothing to curtail growth in oil consumption until shortages occurred. The shortages of 1973 and 1979, exacerbated by partial loss of foreign supply, were followed by accusations of collusion by the oil industry to create shortages.

Nevertheless, the decline in US oil production had begun in 1970 as predicted in 1956 (ref. 1), and it continues inexorably. The United States is now dependent on foreign sources for half of its oil supply and has demonstrated its willingness to wage war to ensure continued access to oil from the Persian Gulf.

The warnings of falling US oil production were based on geological constraints, well-drilling records and petroleum field discovery and production histories. Now, on the basis of analogous data, geologists warn that *global* oil production will peak and begin its permanent decline around the year 2020 (refs 5 and 6). This message is as unpopular today as was its analogue in the 1950s.

US citizens pay much less for gasoline than do people in other industrialized countries, consume more oil each year than those any other country and resist modest increase in fuel taxes, while US car-makers have successfully fought more stringent automotive fuel economy standards. If current US demand for oil persists, increasing dependence on Persian Gulf supply will cause higher oil prices; in time the United States may not be able to afford the oil it demands. Recent history suggests that this could lead to war in the Middle East over the last of the world's oil reserves.

A crisis is commonly a long-term problem not faced soon enough. The United States, by ignoring such problems until catastrophe is at hand, seems willing to believe anything but the truth. A rational long-term view is that permanently and significantly higher fuel prices through government surtax would encourage sustained fuel conservation as well as research on substitutes for conventional oil.

But US political leaders hoping for re-election will not advocate a long-term solution that requires immediate economic sacrifice by their constituents. All voters use crude oil derivatives, and higher oil prices are unpopular. A congress-

man, although aware that higher fuel prices are in the long-term public interest, also knows that they will engender short-term economic hardship without perceived benefit for several elections ahead, and thus lead to electoral disadvantage.

Consider what happened to President Jimmy Carter for acknowledging the severity of the energy problem. Compare this with President Ronald Reagan's popularity, resulting in part from his denial of the energy problem.

If an important but unpopular idea does not receive a fair hearing, public education is the solution. The US government will continue to ignore its energy problem until it escalates to crisis proportions unless a well-informed constituency demands action based on long-term considerations in spite of short-term economic hardship. So far, the American public demands more fuel than any other nation at prices lower than other nations pay. The course of major events during the next three decades could hinge on whether the US people learn that this demand cannot be satisfied much longer.

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Adversarial system in science

SIR — Scientists criticize adversarial systems in law, but, in my opinion, the system has an unrecognized part to play in science. The facts relating to almost any serious scientific problem may usually be interpreted in more than one way. There are also many ways in which to preselect the facts considered. This means that the same data may have several different, and frequently opposite, explanations.

Unfortunately, the ability of the human mind to find an alternative explanation of

facts once a plausible one has been proposed is extremely poor; I call that phenomenon the presumption of the first explanation.

The history of science demonstrates that it may take tens to thousands of years to understand that the same facts may be interpreted in alternative ways. For example, Ptolemy's Earth-centred cosmology had been about for 1,400 years before it was replaced by the Sun-centred system by Copernicus, based on the same facts.

The question for science is how can we shorten these periods of self-deception, when the community as a whole believes that the truth has been already found?

The adversarial system offers a way of solving the problem. It is time to understand that consensus in the scientific community is not a good sign, but rather a symptom of a crisis. The polarization of views is normal.

One of the lessons of history is that nobody can know what is the ultimately proved truth. Any theory is only a viewpoint from which we look at the world. From some viewpoints, more elements are perceptible, from others, fewer. But the greater the number of viewpoints, the richer picture of the real world we have. P. Feyerabend's "proliferation of theories" has to become the way forward for science.

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Pauling's missed Nobel prize

SIR — You say it is a mystery why Linus Pauling did not identify the structure of DNA before Watson and Crick (*Nature* **370**, 584; 1994). Pauling did not think it was a mystery.

He was a passenger on a flight of mine from Miami to San Francisco in the early 1980s when I was a United Air Lines captain. Having once been his neighbour, and having talked to him before (he advised me once while at the check-out counter of the Portola Valley Market to forgo buying a particular brand of toilet paper for another, "it's cheaper and better!"), I went back to talk to him after we settled down in cruise.

I mentioned I had just read *The Double Helix* and, knowing from the book he had been on the same track, I volunteered: "You came very close to being the first recipient of three Nobels". He answered: "Yes, I was weeks away, and if they had not got hold of Franklin's X-rays I would have beaten them to it."

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

The challenge of molecular medicine

The potential impact of molecular biology on medical problems is clear. But as participants at *Nature's* conference on molecular medicine in San Francisco (22–23 September) found, producing practical therapies will not be simple.

San Francisco. Thirty years ago, as Bernard Fields (Harvard) recounted at *Nature's* meeting last week, his interest in infectious diseases was greeted with disdain. Bacterial infections were a thing of the past, thanks to antibiotics, and vaccines had ensured the same was true of viral diseases such as polio; surely he could make better use of his time?

Other talks made it very clear (if anyone had any doubts) how wrong these pundits were. But as well as the resurgence of infections that man, in his hubris, had long thought conquered, the intervening years have seen a deepening in our understanding of disease brought about by the advent of molecular biology. And perhaps, as David Baltimore (MIT) remarked, it is not unreasonable that techniques developed in the search for knowledge should take 20 years to produce a workable medical technology.

Nature has of course already had at least one related conference (see *Nature* **366**, 505; 1993). But on that occasion it was the search for molecular models and tools that dominated, rather than (as here) the search for insights that might one day yield effective therapies. This transition can only become more important as time goes on, and is reflected in the impending launch of *Nature's* latest sister journal, *Nature Medicine*. It has also prompted at least one graduate school to introduce a new curriculum, aimed at producing tomorrow's biomedical researchers (Donald Ganem, University of California, San Francisco (UCSF)).

In many cases, though, the transition is still at a surprisingly early stage. Tuberculosis is undergoing a worldwide resurgence, and is again a leading cause of death. Only the advent of the knockout mouse, however, has established that interferon γ , β_2 -microglobulin and tumour necrosis factor α are all necessary for successful defence against the infection (Barry Bloom, Albert Einstein College of Medicine, New York). While this puzzling constellation may reflect the role of nitric oxide in killing bacilli ingested by macrophages, it underlines our poor understanding of the disease's pathogenesis. But the finding that Hispanics living in the Bronx in New York are as much at risk of tuberculosis as AIDS patients suggests that improved housing, sanitation and occupational health could be of major benefit even in developed countries.

Similar problems arise in the study of leishmaniasis, another major scourge in developing countries. Here, the puzzle is to understand why some infected individuals, like certain strains of mice, mount an ineffect-

ive immune response and succumb to systemic infection (Richard Locksley, UCSF). At least in mice, the answer appears to be that the immune response is dominated by the T_H2 lymphokine profile appropriate for extracellular parasites, rather than the T_H1 profile needed to eliminate intracellular parasites such as leishmania.

In other cases, it is the infectious agent itself that is new. One example is the recent outbreak of hantavirus in the United States (see *Nature* **370**, 409; 1994). Another is the necrotizing fasciitis recently dramatized by the media, which may be caused by streptococci that elaborate exotoxins previously thought to be confined to staphylococci (Richard Krause, Fogarty International Center, National Institutes of Health).

But the need for a better understanding of pathogenesis is by no means confined to infectious diseases. The finding that ICAM-1 and VCAM-1 are the only molecules responsible for monocyte adhesion to the arterial endothelium in the initial stages of atherosclerosis, for instance, surely suggests new therapeutic approaches (Russell Ross, University of Washington, Seattle). The key role of platelet-derived growth factor in the subsequent cellular proliferation may point to another target.

Our understanding of cancer, of course, has been more affected by molecular biology than that of any other condition, and recent developments — the possibility of screening shed cells for early detection of malignancy, for example (David Sidransky, Johns Hopkins University, Baltimore), or the isolation of the first gene responsible for hereditary predisposition to breast and ovarian cancer (Mary-Claire King, UC, Berkeley) — have already been covered in *Nature* (**369**, 13 & **371**, 279; 1994 respectively).

Even here, though, there were surprises. Primary tumours have long been known to suppress the growth of metastases, but the discovery that they do so by secreting an inhibitor of angiogenesis suggests new approaches to both diagnosis and therapy (Judah Folkman, Harvard). The possibility that not all tumours with unstable di- and trinucleotide repeats may have defects in mismatch repair, too, may have diagnostic implications (Paul Modrich, Duke University, Durham, North Carolina). But in other cases, such as cancer cell apoptosis, the phenomenon in question is so complex that it will be some time before research affects medical practice (Stanley Korsmeyer, Washington University, St Louis).

Sometimes the flow is reversed. Our

understanding of signalling by the T-cell receptor, for instance, has been greatly enhanced by the study of immunodeficient patients (Arthur Weiss, UCSF). And the account by David Nathan (Harvard) of his work on thalassaemia over 25 years was a beautiful example of the fruitful interplay between molecular understanding and clinical care.

But in general, it is the tools with which to apply molecular insights that are in short supply. The most obvious, perhaps, is gene therapy, but as Inder Verma (Salk Institute, San Diego) remarked, this is beginning to resemble an onion — the more you explore it, the more tears you get. In particular, although adenovirus vectors have many advantages that retroviruses lack (notably growth to high titers and an ability to infect non-dividing cells), host immune responses seem likely to make them virtually unusable *in vivo*. Modifying cells in culture before returning them to the host (the so-called *ex vivo* approach) avoids these difficulties, and has so far had more promising results; suitably modified fibroblasts (and perhaps neurons) can even trigger regeneration of cholinergic neurons in the brains of mice and monkeys (Fred Gage, UC, San Diego).

Where genes cannot form the basis of therapy, drugs must suffice, and there has been no lack of attempts to develop them. Sometimes, research suggests a new target, like the farnesylation reaction used to attach the Ras oncoproteins to the cell membrane (Guy James, University of Texas Southwestern Medical Center, Dallas). On other occasions, known structures can serve as models for therapeutically important targets, allowing computerized selection of possible inhibitors (Fred Cohen, UCSF). Modelling of the malarial cysteine protease on the basis of related enzymes from kiwifruit and papaya has already uncovered at least one compound with promising antimalarial activity in mice.

More radical departures from traditional pharmacology may eventually have an even greater impact. Procedures for screening random oligomer libraries on the basis of their biological activity (so-called combinatorial chemistry), for instance, should eventually allow rapid isolation of (ant)agonists directed at almost any biological target (Paul Bartlett, UC, Berkeley). But whatever the exact route by which they are produced, it is clear that the long wait for medical applications of molecular biology will soon be over. The result can only be a steady stream of advances in patient care. **Nicholas Short**

Cosmological conflict

Craig J. Hogan

THE Hubble constant, the ratio of recession velocity to distance for galaxies in the distant expanding Universe, has at last been estimated from a direct calibration of the distance to the Virgo galaxy cluster. But the new estimate of $H_0 = 87 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1}$ given by Pierce and colleagues on page 385 of this issue¹ is high enough to conflict with some other recent measurements, and will come as unwelcome news to many theorists. Plugged into their favoured models, it makes the Universe considerably younger than the estimated ages of many stars.

For decades, astronomers have struggled with establishing the scale of the distant Universe. A critical step in this programme is to find reliable distances of the nearest few galaxy clusters, such as Virgo and Fornax, whose distance ratios to very distant clusters have been firmly established by a variety of other methods^{2,3}. Pierce *et al.*¹ have measured brightness and periods, and hence distance (14.9 ± 1.2 megaparsec), for three Cepheid variable stars in galaxy NGC4571 in the core of the Virgo cluster, establishing for the first time a cluster distance based on a well trusted indicator. Their measurement immediately translates into an estimate for the Hubble constant.

Virgo distance

Cepheid variables are bright stars whose brightness varies periodically, on time-scales between 1 and 100 days. The period of a Cepheid is very tightly correlated with its brightness, so they are excellent indicators of distance. Cepheids have always figured prominently in the extragalactic distance scale, first establishing that other galaxies lay outside our own, later giving the first estimates of the Hubble constant using nearby galaxies. As technology has improved, ever more distant Cepheids have been measured, allowing direct distance measurements farther out in the 'Hubble flow'.

The pivotal importance of Virgo Cepheids for the cosmic distance scale has been recognized for years. But they are very far away — twice as far as the most distant previously measured Cepheids — and have resisted measurement from ground-based telescopes, because they are faint stars in crowded fields, a situation where the blurring caused by the Earth's atmosphere is a major handicap. Measuring Virgo Cepheids has always been one of the central goals of the Hubble Space Telescope (HST).

It is all the more impressive then that Pierce and colleagues have managed the feat from the ground. They were aided by the good seeing at Mauna Kea, by the

superb linearity of modern charge-coupled device detectors, and by a sophisticated instrument at the Canada-France-Hawaii telescope. This device, called HRCam, further improves images using moving ('adaptive') optics which actively remove image motions on time-scales as small as 0.01 s. Although some atmospheric blurring remains, this 'tip-tilt' correction offers enough improvement in image quality to make the critical leap to Virgo distance.

Is the distance debate over? Do we now really know the Hubble constant to 8 per cent accuracy? Not quite, as a swirling debate will surely soon testify. The first issue to come up will be whether the galaxy NGC4571 is really in the core of the Virgo cluster. If it is well in front of Virgo, then a measurement of its distance is not a measurement of the cluster distance at all.

But there are good reasons to believe that it is in the Virgo core. The gas in NGC4571 shows signs of considerable stripping, typical of spiral galaxies that are moving through the hot, relatively high-pressure gaseous environment of a galaxy cluster. Even more suggestive, the velocity of NGC4571 is much lower than that of the Virgo cluster, whereas galaxies in the vicinity but on the near side of the cluster (and not in the core) should be falling into Virgo, therefore receding from us faster. The low velocity is naturally explained if NGC4571 is on a typical orbit for the core of the cluster, and happens to be coming towards us at the moment with a typically high cluster core velocity. In this case, even if it is on the near side of the core, its distance should be within about 10 per cent of the cluster mean. These issues are sure to focus attention on the detailed dynamics and the evidence for subclustering in the Virgo region. Such studies are likely to enlarge the estimated errors on H_0 even if the estimated distance to NGC4571 remains intact.

We will not need to wait long for the debate to move forward. The HST team⁴ has already announced the detection of Cepheids in another Virgo cluster galaxy, M100, and their distance estimate is imminent. Eventually, with a sample of Cepheid distances to several Virgo galaxies, a variety of new tests will become possible which are independent of the cluster membership problem, based on galaxy-by-galaxy calibration of several other distance indicators, such as the Tully-Fisher method, the planetary nebula luminosity function and the surface brightness fluctuation method. These are precise techniques for measuring distance ratios between galaxies, whose absolute calibration has previously been based on

only a small number of local galaxies.

The large Hubble constant derived here agrees within the errors with these other independent methods as usually calibrated, which usually yield^{2,3} estimates around $H_0 = 80 \pm 10 \text{ km s}^{-1} \text{ Mpc}^{-1}$. It also agrees within the errors with the 'expanding photosphere method' distances to type II supernovae, which yields⁵ $H_0 = 73 \pm 6 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (including an estimate of both statistical and systematic errors). Such distances are derived directly from a physical model of the supernova atmospheres, and are independent of any other distance calibration. Although some other 'direct' techniques (using hot gas in galaxy clusters and time delays between gravitational lens images of quasars) currently yield lower estimates of H_0 , there is probably no contradiction at present within the suspected systematic errors of these methods.

Contradictions

However, the new result directly contradicts the conclusion from new HST measurements of Cepheid distances to two other galaxies closer than Virgo, which were used to calibrate the brightness of three type Ia supernovae to obtain^{6,7} $H_0 = 52 \pm 9 \text{ km s}^{-1} \text{ Mpc}^{-1}$ from the Hubble diagram of more distant Ia supernovae. The resolution of this sharp conflict is not clear, but will be tested soon. One possibility is that the particular supernovae used for local calibration are unusually bright compared to the typical distant type Ia, which would lead to an exaggerated estimate of the distance of the distant sample and hence a low H_0 estimate. Evidence for this can be sought in the archival supernova light curves, because the decay time of a supernova is correlated with its brightness. This possibility will also be tested as more supernova Ia distances are obtained with the HST.

The high value of H_0 has a significant impact on cosmological theory, mostly unwelcome to the conservative theorist. For a start, a high H_0 creates difficulties with structure formation theories, as it decreases the characteristic scale associated with the epoch at which the Universe becomes matter-dominated; thus the predicted coherence scale of cosmic structure is reduced below that observed.

The biggest problem however is with the age of the Universe, predicted for the favourite, geometrically flat, matter-dominated universe to be $2/(3H_0)$ or 7×10^9 years for $H_0 = 87 \text{ km s}^{-1} \text{ Mpc}^{-1}$, only

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about half the shortest astrophysical estimates of the oldest stellar ages. Even an open universe model cannot completely remove this discrepancy, forcing either a revision of age estimates or introduction of a 'cosmological constant' into the equations of motion of general relativity — the equivalent of self-gravitation of empty

space. With such important consequences resting on the outcome, the debate looks set to continue. □

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IMMUNOLOGY

How to stop bad B cells

G. J. V. Nossal

LYMPHOCYTE traffic patterns are a closed book to most immunologists, who prefer the simpler world of *in vitro* models of signal transduction and lymphocyte activation. Now the reductionists will have to sit up and take notice — the paper by Cyster *et al.* on page 389 of this issue¹ reveals a novel and surprising 'homing' disability in self-reactive B cells, uncovering a new and subtle layer of regulation among the mechanisms that keep us all from succumbing to autoimmune diseases. B cells with specificity for a soluble, circulating self antigen do not manage to reach a microenvironment (known as a lymphoid follicle) which appears to be a safe haven promoting health and longevity. Instead, they knock on the door, and, having been refused entry, despair and die. Interestingly, the impedance to crossing the threshold is relative, not absolute. The 'bad' anti-self B cells are jostled aside by 'good' anti-foreign B cells which out-compete them and occupy the follicle. Arrange matters such that there are no anti-foreign B cells, and the anti-self B cells get where they want to go.

The beautiful and intricate micro-architecture of lymphoid tissues such as lymph nodes and spleen has been a source of fascination for over a hundred years. Yet, to attempt to understand it, it was necessary to tear it to bits. Only then could we come to grips with the amazing heterogeneity of lymphocytes. Studies on single cells, cloned lymphocyte lines, and populations of lymphocytes in short-term tissue culture or injected into other mice have underpinned cellular immunology. A great deal has been revealed thereby — the 'one cell—one antibody' rule; proof that antigen selects monospecific lymphocytes from a large, diverse repertoire; the division of lymphocytes into the two great families of T and B cells; the nature of the help that T cells give to B cells; and the chief cellular events of immunological tolerance. We owe a great debt to the hardy minority of scientists who kept

struggling with 'real life' lymphoid organs at the same time. The black box gradually yielded to imaginative use of autoradiographic and immunohistological approaches.

An early victory was the discovery that lymphoid follicles, spherical aggregations of small B lymphocytes and specialized

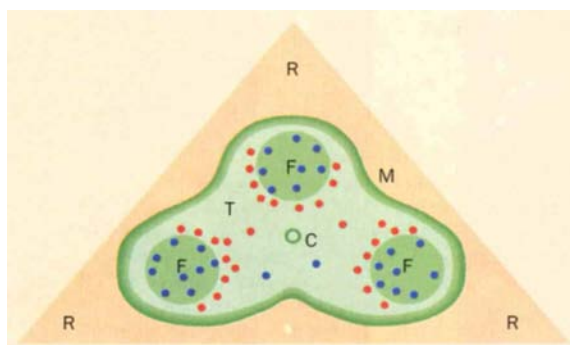


Diagram of a section of murine spleen. C, central arteriole of the white pulp; T, T-cell and migration area; F, primary lymphoid follicle; M, marginal zone; R, red pulp. Blue circles show the location of normal B cells in the presence or absence of antigen, or of anergic B cells if not impeded by competition from normal B cells. Red circles show the location of anti-self B cells in the continued presence of their self antigen, if normal B cells are present to give them some competition.

follicular dendritic cells (FDC), capture antigen in a curious way. Antigen is held extracellularly on the surface of FDC processes for many months, permitting ready interaction with surrounding B cells. It gradually became clear²⁻⁴ that the follicles represent a specialized microenvironment for the creation of memory B cells. Shortly after antigen injection, activated B lymphocytes migrate into the follicle; they then proliferate extensively (creating what is called a germinal centre), hypermutate their immunoglobulin heavy- and light-chain variable (V) genes, move towards the antigen-laden FDC, and either receive a positive selective stimulus if their affinity for antigen has been raised through mutation, or die locally by apoptosis. The new findings¹ show for the first time that the lymphoid follicles also have profound effects on 'virgin', unactivated B cells.

The centre point of these studies is in-

telligent exploitation of transgenic models developed by the group to study immunological tolerance⁵. It had previously been shown⁶ in normal mice that B lymphocytes can be rendered tolerant (non-reactive to a specific immunogenic stimulus) in one of two ways: by deletion of the specific B cell, or by a functional inactivation, that is, the induction of clonal anergy. If mice are rendered doubly transgenic so as to express a high-affinity, anti-hen egg lysozyme (HEL) receptor on most of their B cells, and also to secrete HEL into the serum and lymph, all the B cells become anergic. These B cells circulate and home to lymphoid organs normally, though having a shortened lifespan.

It was felt that such experiments were unphysiological, given the vast preponderance of the transgenic anti-HEL (anti-self) B cells. So Cyster *et al.* created various models in which the anti-self population was in the minority. These included injecting mature anergic cells into other mice with a normal B-cell pool; creating mixed populations of transgenic and normal B cells through a double bone-marrow transplant; or constructing a situation where the B cells carried a transgenic heavy chain but a normal, endogenously assembled, light chain, with the result that only 1 per cent of B cells ended up as having high affinity for HEL. In each case, the behaviour of the B cells was monitored in the absence of the neo-self antigen HEL, in which cases the B cells all behaved similarly, or in the presence of transgenic HEL. The three models led to the same conclusion, summarized in the figure.

Although normal B cells enter the spleen and migrate through the T-cell areas, their innate tendency is to home to the primary lymphoid follicles, which are predominantly B-cell areas with only a few scattered T cells. Some further maturation occurs here locally, as evidenced by phenotypic changes at the cell surface. In the case of transgenic animals where all of the B cells are anti-HEL, this same migratory pattern is evident whether HEL is present or not. However, if a majority of the B cells are not anti-HEL, but represent a repertoire of diverse specificities as is normally the case, the normal cells in some way prevent the anti-self B cells from reaching their preferred destination — but only if the self antigen remains in the environment. It is almost as though the self antigen were a lead weight on the B cell, preventing it from running fast enough. There are clearly limits to the number of B cells which can be fitted into the preferred, maturation-enhancing environment. So the anti-self B cells briefly pile up just

outside the follicle, but soon die.

How do these contrived transgenic experiments relate to normal B-cell physiology? It has long been known⁷ that the bone marrow produces far more B cells than can be accommodated in the recirculating and long-lived⁸ B-cell pool. Presumably, recently created normal B cells must also compete to gain entry into the maturation-inducing and longevity-promoting follicular environment. The most dangerous anti-self B cells, those with reactivity against ubiquitous cell-surface structures such as self major histocompatibility complex antigens, are eliminated by deletion at the very moment of birth in the bone marrow⁹. As the generator of antibody diversity is random, this leaves many B cells of lower affinity for various self antigens, which escape from the marrow. The mechanism described by Cyster *et al.* prevents them from being a potential cause of autoimmunity because it ensures their premature death.

Monumental challenges remain. What promotes normal B cells reaching the follicles? If, as Cyster *et al.* suggest, "access to a trophic factor present in the follicular microenvironment" is the operative variable, then what might that factor be, and how is access to it limited by occupancy of a B cell's receptors by its antigen? What is biochemically different within the anergic cells? What factor(s) within the follicular microenvironment promote the phenotypic and functional changes within a B cell that permit the B cell, still antigenically inexperienced, to mature and eventually leave the follicle and become a long-lived recirculating B cell? What are the relationships, if any, between these newly defined properties of the follicular microenvironment, exerted on the primary B-cell repertoire, and the more established properties that make the same area the chief and perhaps the only site of V-gene hypermutation following activation of B cells?

In the first instance, it is urgent to examine both primary follicles and germinal centres for the expression of all the genes that might be involved. These include genes encoding all the cytokines, co-stimulatory ligands and their receptors, and the various families of adhesion molecules. Reductionism will doubtless have to enter again, in the form of attempts to re-create the follicular microenvironment *in vitro* from its consti-

tuent parts, including T cells, B cells, FDC and localized stromal cells.

It is worthwhile reflecting how far we have come since clonal deletion or repertoire purging became the dominant way of explaining tolerance to self antigens. We now realize that killing specific lymphocytes in immature animals would have been a blunt weapon indeed. We recognize the influence of immaturity not only of the immune system as a whole, but of the individual cell. We acknowledge that geography is important, organs such as the thymus and the bone marrow possessing special mechanisms to censor potentially self-reactive cells. We have noted both escape mechanisms from tolerance induction, and back-up, fail-safe mechanisms that cut in to limit possibilities for

autoimmunity¹⁰. We see clonal anergy, a potentially reversible functional impediment of a cell's immune response capacity, as a more subtle control mechanism than outright cell death.

We must now come to grips with the fact, revealed by Cyster *et al.*¹, that the behaviour and fate of a single anergic cell can be influenced by other normal cells. As each of these concepts becomes embedded in immunological folklore, we will be freed to probe still further the rules governing immunology's most abiding problem: self–nonself discrimination. □

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ASTROPHYSICS

New handle on a maser pump

Philip R. Jewell

In the early years of laboratory lasers, the devices were sometimes termed 'solutions in search of a problem'. Astronomical masers, first identified^{1–3} in 1965, have attracted similar or still less flattering descriptions. Just as lasers soon found vast use in industrial applications, so now are astronomical masers proving their worth. The report by Miyoshi *et al.* on page 395 of this issue⁴ demonstrates the potential of SiO masers as an astrophysical diagnostic and answers a lingering question about their operation.

Whereas man-made lasers have become well understood and are a common feature of everyday life, certain aspects of nature's versions have remained a mystery. The principal prerequisite for maser action, whether cosmic or man-made, is an inversion of the energy-level populations⁵. In most environments, the populations of atomic or molecular energy levels tend towards thermal equilibrium. To make a maser, this natural tendency must be subverted and population distributions turned on their heads. This process is known as pumping the maser.

In astrophysical cases, most pump mechanisms require special circumstances such as velocity gradients or line saturation effects to create the right conditions for inversions. For example, in the case of the well understood 1,612-MHz OH maser, the molecules are excited into high-lying states by infrared radiation from ambient dust. If the radiative decay transitions are saturated, the upper levels of some states are over-populated, and a maser can arise⁵. The agent that pumps the molecules into high-energy states is usually radiation or collisions, but it can be difficult to tell which, and the SiO maser pump has been a particularly diffi-

cult one to classify; over the past ten years, viable models have been proposed for both radiative and collisional pumping.

What Miyoshi and co-workers have done is to devise an accurate observational test to discriminate between radiative and collisional pumps. Because of the large differences in energy between the SiO vibrational levels, a radiative pump is unlikely to produce masers arising from different vibrational states in the same body of gas. Collisional pumps, on the other hand, can produce masers from different vibrational states in the same volume of gas rather easily⁶. Using very-long-baseline interferometry (VLBI) between the Nobeyama and Kashima telescopes, the team simultaneously imaged rotational emission from two vibrational states separated by an energy equivalent to about 1,700 K. The simultaneous observations minimize atmospheric and other systematic phase errors that make separate observations difficult to compare. Miyoshi's group found that the emission from the two vibrational states was nearly coincident on the sky, indicating that the masers arise from the same gas. This evidence weighs very heavily in favour of collisional pumping.

As a beneficial consequence of the pump model work, Miyoshi and colleagues' experiment has demonstrated how useful the SiO maser can be as a diagnostic tool for an important astrophysical problem. Most SiO masers reside in the 'extended atmosphere' of highly evolved giants and supergiants⁷. Stars of this type lose copious amounts of their stellar envelopes as a wind, enriching the interstellar medium and altering the course of their own evolution. These objects are, in fact, the principal source of

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recycled interstellar matter in the Galaxy. Although the mass loss is observed to happen, how it happens is not well known, and until now few observational tools have been available to solve the problem. From Miyoshi and colleagues' observations, and other recent results from VLBI⁷, it seems that the SiO maser will be most useful in this role — it is known to originate within a few stellar radii of the photosphere, in the region where material is probably being accelerated away from the star. As the masers appear nearly point-like on VLBI images and also can provide velocity information through their spectroscopic line profiles, it appears likely that the motion of cells of gas can be traced as they flow through the extended atmosphere.

This joins a growing list of important discoveries made possible by maser observations. In years past, critics have pointed out the difficulties of deriving traditional spectroscopic information from maser research. In the simplest cases of thermal emission, the intensity of the observed line profile is directly proportional to the number of emitting atoms or molecules along the line of sight. By observing two or more transitions of a thermal species, one can often derive its density and temperature. In contrast, masers arise from a nonlinear amplification of emission; the emission is often not isotropic and the intensity can be strongly affected by clumps or loss of velocity coherence in the emitting gas, so in general, one cannot derive densities and temperatures with any useful accuracy. This realization led some to reject maser emission as an astrophysical tool, a view akin to condemning a hammer because it cannot also be used as a screwdriver.

When used to their proper advantage, masers can be powerful beacons, delineating structures and tracing gas motions. They have been used in proper motion experiments to identify the source of stellar outflows, provided rotation curves near the Galactic Centre, measured the sizes and distances of circumstellar envelopes, and identified massive star-forming regions in distant galaxies. In many such cases, masers have demonstrated their utility as powerful astrophysical tools. □

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Fishy tales of kidney function

Clive Palfrey and Andrew Cossins

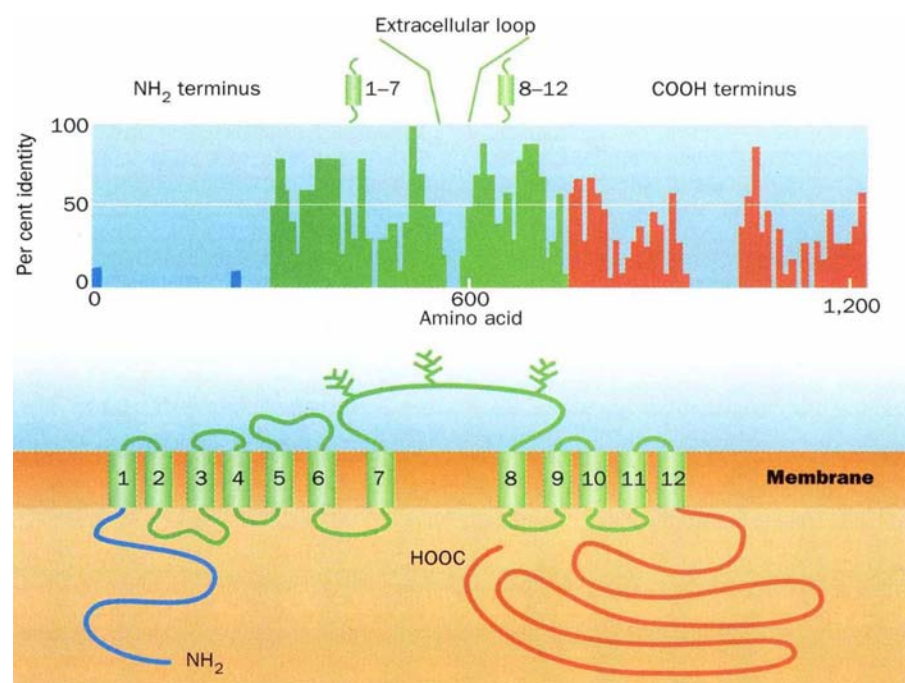
MOVING salt is perhaps the most ancient osmoregulatory function of animal cells and has led to the evolution of highly efficient salt-transporting epithelia. Vectorial salt transport often involves powerful cotransporters that work in conjunction with pumps and channels. In contrast to the remarkable progress in characterizing these pumps and channels at a molecular level, the electrically silent cotransporters have proved much more difficult to crack. Three recent papers¹⁻³ change this by describing the primary structure of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ and Na^+/Cl^- cotransporters in mammalian kidney and by making it clear that these proteins represent a new transport protein family of unusual diversity and distribution.

The cotransport of Na^+ , K^+ and 2Cl^- (NKCC) was originally identified in Ehrlich ascites cells and avian erythrocytes but is now known to be widely distributed⁴. In non-epithelial cells it plays a role in volume control, particularly the phenomenon known as regulatory volume increase, whereby cells regain their original volume after shrinkage. This cotransporter is better known, however, for its central role in net epithelial salt transport⁴. In both absorptive and secretory epithelia it functions to raise intracellular Cl^- above electrochemical equilibrium so that the ion can escape

across the opposite border of the cell, usually through a conductive Cl^- channel.

Blood salt level is clinically manipulated using diuretics such as the thiazides and sulphonylbenzoates (bumetanide for example). Their potency is due to a powerful inhibition of Na^+/Cl^- and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport, respectively, in the kidney tubule. Although radiolabelled bumetanide derivatives allowed the identification of a bumetanide-binding component of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter⁵, little further information was forthcoming, partly because the cotransporter was functionally inactive after reconstitution. But monoclonal antibodies against the bumetanide-binding protein from shark rectal glands⁶ were later used to immunoscreen an expression library from the gland and eventually a full-length complementary DNA encoding a 'secretory' form of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC1) was cloned¹. Transfection of human cells with shark cDNA led to expression of a bumetanide-sensitive cotransport (BSC) activity which was activated by reduction in cellular Cl^- levels. A closely related form of the cotransporter, NKCC2, has now been discovered in mammalian kidney and presumably mediates salt absorption in the thick ascending limb of Henle's loop².

Another approach to cloning these



Structure of salt cotransporters, showing twelve membrane-spanning helices and cytoplasmic N- and C-terminal domains. The percentage identity of the six cloned Na^+/Cl^- and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters^{1-3,8,9} is compared (top) and shows the transmembrane domains to be highly conserved. (From data provided by S. C. Hebert and E. Delpire.)

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transporters also started with fish tissue: flounder urinary bladder expresses a thiazide-sensitive cotransporter (TSC) of Na^+ and Cl^- (ref. 7) which is also found in the early distal tubule of mammalian kidney. Expression of flounder bladder messenger RNA in *Xenopus* oocytes resulted in the cloning of a cDNA encoding a 1,002-amino-acid Na^+/Cl^- cotransport protein⁸. Probes derived from this flounder cDNA have now been shown to bind mRNAs of different sizes in mammalian kidney³: a 4.5-kilobase cDNA was found by expression cloning in oocytes to code for a TSC (rTSC) and a 4.6-kilobase cDNA to code for a $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (rBSC1).

One major benefit of this work is to improve our understanding of cotransporter diversity and distribution in relation to tissue function. *In situ* hybridization in kidney shows that rTSC is located predominantly in the outer cortex and that rBSC1 binds to the medulla³. Both patterns are consistent with the recognized functional assignment of the two transporters to the distal tubule and the thick ascending limbs, respectively. Intriguingly, Payne and Forbush² have found a cassette exon of the mammalian renal NKCC2 gene that is alternatively spliced to yield three isoforms. These are differentially distributed within the kidney, one to the cortex, another to the medulla and the third over both regions.

Other members of the family should soon be forthcoming. Screening of tissues other than kidney reveals a 7-kilobase mRNA which may represent the predominant non-epithelial form of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport. Cloning of this isoform (BSC2) shows it to be the mammalian counterpart of NKCC1, which is expressed in a wide variety of tissues and maps to a different chromosome from BSC1 (ref. 9). In addition, significant homologies have been found with *Caenorhabditis elegans* and cyanobacterial sequences¹⁻³. Screening for variants also offers the chance to address several interesting questions, including whether there is a defect in $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport in some types of hypertension¹⁰. Attention will focus as well on the K^+/Cl^- cotransporter that is stimulated during regulatory volume decrease (the shrinkage to normal volume

after swelling) in many cells and whose activity may be regulated in a reciprocal coordinated way with the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter¹¹. It is weakly inhibited by the bumetanide class of diuretics, which suggests some structural homology with the BSC class of cotransporters.

Although all the salt cotransporters identified so far have twelve potential membrane-spanning helices, they show little sequence similarity with other members of the dodecahelix transporter superfamily³, including the amine transporters that are coupled to Na^+ and Cl^- . The transmembrane domains of the TSCs and BSCs are highly conserved (see figure), but their long cytoplasmic carboxy and amino termini are variable and probably function as regulatory domains and attachment sites for other proteins. Con-

sensus sites for various protein kinases have been identified^{1,3} and phosphorylation of at least some of these may be central to cotransporter activation. Although there is a reasonable understanding of the sequence of events during hormone-induced stimulation of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport, there is virtually no information on how the cell volume signal is transduced. Identification of these volume-sensitive signalling pathways remains a major challenge. □

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SIGNAL TRANSDUCTION

Regulating S6 kinase

Julian Downward

THERE has been enormous progress in understanding how Ras proteins control cell proliferation and, following oncogenic mutation, malignant transformation¹, and at times it has seemed that they might be able to carry out just about any regulatory task imaginable. Ras is the veritable superhero of intracellular signalling — but hero turned villain when led astray by carcinogenic insult. Even Ras has its limitations, however. On page 426 of this issue², Ming *et al.* show that control of $\text{p70}^{\text{s6k}}/\text{p85}^{\text{s6k}}$, a mitogen-regulated kinase specific for ribosomal protein S6, is through a hitherto unknown Ras-independent pathway. This work, together with that of Chung *et al.*³ published in July, provides new insights into the regulation of this critical kinase.

Two distinct families of kinases have been identified that phosphorylate S6 *in vitro*. One of them, the *rsk*-encoded kinases of relative molecular mass 85–92K, is controlled by the Ras–MAP kinase pathway, but does not seem to phosphorylate S6 in intact cells. The other, which consists of the *s6k* alternative splice products, cytosolic p70^{s6k} and nuclear p85^{s6k} , is responsible for S6 phosphorylation in intact cells. Microinjection of quiescent cells with neutralizing antibodies against p70^{s6k} and p85^{s6k} blocks their ability to progress through G1 into S phase in response to serum^{4,5}, suggesting that $\text{p70}^{\text{s6k}}/\text{p85}^{\text{s6k}}$ (henceforth referred to as S6K) plays a key role in cell proliferation. Furthermore, the inhibition of cell-cycle progression by the immunosuppressant rapamycin correlates well with its ability to inhibit the activation of S6K (refs 6, 7). One result of S6 phosphorylation by S6K is that ribosomes preferentially translate a

family of messenger RNAs containing a polypyrimidine tract at their 5' end⁸; these could encode proteins needed for progression through G1.

S6K is activated by treatment of cells with a wide variety of mitogens. It becomes phosphorylated on a number of serine and threonine residues, several of which are followed by proline residues, pointing to the immediate upstream involvement of a proline-directed protein kinase⁹. Because S6K is activated in Ras-transformed cells¹⁰, it had been speculated that it may lie on a signalling pathway directly triggered by Ras. Ming *et al.*² now show that dominant negative mutants of Ras or of the serine/threonine kinase Raf, a downstream effector of Ras, both fail to inhibit S6K activation by epidermal growth factor (EGF) or phorbol ester. By contrast, MAP-kinase activation by these stimuli is inhibited by the Raf mutant, while dominant negative Ras inhibits its activation by EGF but not by phorbol ester. The degree of MAP-kinase inhibition by dominant negative Ras depends on the cell type studied^{11,12}.

So Ras is not necessary for S6K activation. But is it sufficient? To address this question, mutants of the receptor for platelet-derived growth factor (PDGF) have been used that lack the kinase insert region: this is a sequence within the kinase domain that is not necessary for enzymatic activity but which contains a number of tyrosine autophosphorylation sites that dock SH2-containing proteins¹³. PDGF receptor lacking the kinase insert still activates Ras normally, probably through binding of a complex of the phosphatase Syp, the adaptor protein Grb2 and the guanine-nucleotide-exchange factor Sos

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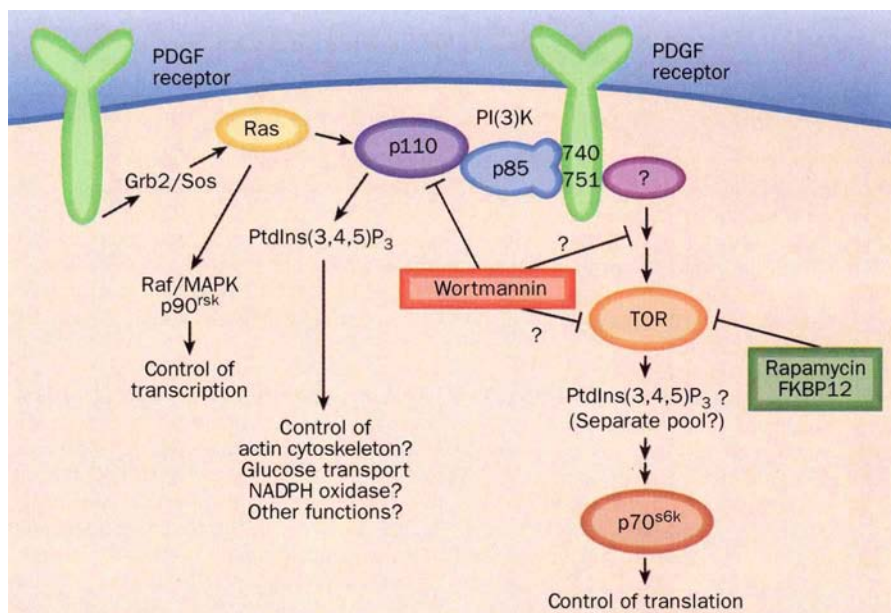
to tyrosine 1,009 near the carboxy terminus of the receptor¹⁴. However, this mutant is unable to activate S6K, implying that Ras is not in any way involved in the regulation of this kinase.

The work by Chung *et al.*³ implicated phosphatidylinositol-3-OH kinase (PI(3)K) as a mediator of S6K activation by PDGF and insulin. Mutation of PDGF receptor tyrosine residues 740 and 751 together blocks binding of the 85K subunit of PI(3)K and also prevents activation of S6K in response to PDGF. In addition, the PI(3)K inhibitors wortmannin and LY294002 block PDGF and insulin-mediated S6K stimulation^{3,15}.

The simplest synthesis of the data from these two papers — that PDGF activates S6K through PI(3)K but not through Ras — does not at first glance mesh with the observation that Ras contributes to the activation of PI(3)K by directly interacting with the catalytic p110 subunit^{16,17}. Further confirmation that things are not as simple as they might have seemed emerges from the observation of Ming *et al.*² that single mutation of the PDGF receptor at tyrosine 740 fails to inhibit S6K activation but completely blocks PI(3)K binding, whereas mutation of tyrosine 751 blocks both. This is an indication that the heterodimeric p85/p110 PI(3)K that associates stably with the PDGF receptor (and binds Ras-GTP) might not be the molecule responsible for activating the S6K pathway, but that another SH2-domain protein is involved which binds exclusively to tyrosine 751, such as Nck for example¹⁸.

If, given this, one speculates that p85/p110 PI(3)K might not be involved in S6K activation, how could the effects of wortmannin and LY294002 be explained? Wortmannin, the best studied of these PI(3)K inhibitors, inhibits a number of protein kinases at higher concentrations, but is thought to be specific for PI(3)K at lower doses. Even so, it may be unwise at this stage to dismiss the possibility that wortmannin could be exerting its effects through molecules other than PI(3)K. Another possible uncertainty stems from the identification of an ever-increasing number of PI(3)Ks: at least eight have been found in mammals so far, probably only three of which can form stable complexes with receptor tyrosine kinases (M. Waterfield, personal communication). How many of these are wortmannin-sensitive is not known, although wortmannin is known to inhibit a G-protein $\beta\gamma$ -stimulated, non-receptor binding PI(3)K (ref. 19), and even inhibits a phosphatidylinositol-4-OH kinase in fission yeast²⁰. It is possible that a non-receptor binding PI(3)K is responsible for mediating the effects of PDGF receptor stimulation on S6K activation.

A good candidate for such a protein might be the recently cloned mammalian



Possible pathways for p70^{S6K} regulation by the PDGF receptor not involving Ras or the heterodimeric p85/p110 phosphatidylinositol-3-OH kinase (PI(3)K). Only tyrosine 751 is required for activation of p70^{S6K}, as opposed to both tyrosines 740 and 751 for association with p85. TOR (for target of rapamycin; also called RAFT1 and FRAP), is required for p70^{S6K} regulation; it may possess PI(3)K activity and is a possible target for wortmannin. PtdIns(3,4,5)P₃, phosphatidylinositol trisphosphate.

target of rapamycin (TOR), which was previously characterized in yeast^{21–23}. A complex of rapamycin with its binding protein FKBP12 interacts with, and inhibits the function of, TOR. It is not yet known whether TORs possess PI(3)K activity, let alone whether they are wortmannin-sensitive, but they do bear strong sequence similarity to the lipid kinase domain of p110 α and β and Vps34, a yeast PI(3)K involved in membrane trafficking²⁴. Because rapamycin acts on TOR and inhibits S6K activation, TOR has already been firmly implicated in the regulation of S6K. It is possible that TOR is the wortmannin-sensitive PI(3)K that acts downstream of PDGF receptor to stimulate S6K, in which case there would have to be compartmentalization of different PI(3)Ks: the magnitude of the 3' phosphorylated phosphoinositide production in the different compartments could be widely different. A further problem is posed by that fact that phorbol ester

activation of S6K is inhibited by rapamycin but not by wortmannin³: this is evidence against the two inhibitors having the same target, unless the pharmacological activation of protein kinase C by phorbol ester is sufficiently potent and long-lived to overcome TOR inhibition by the rapidly hydrolysed wortmannin. A speculative model of these interactions is shown in the figure.

Whatever form of PI(3)K, or other wortmannin-sensitive molecule, turns out to be involved in the regulation of S6K, it is clear that a number of components remain uncharacterized, not least of which is the direct binding protein for the 3'-phosphorylated phosphoinositides. There is no doubt that this fascinating pathway will keep students of signal transduction busy for some time to come. □

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Smudging the fingerprints

Thomas R. Karl

If scientific knowledge is loosely defined as 'understanding gained by the observation and classification of facts so as to be able to verify hypotheses', then scientific understanding of the climate effects of increasing greenhouse gases now depends on whether we can confirm projections of climate change. Karoly *et al.*¹ have now taken us a step closer to this goal in a prime example of the so-called fingerprint detection strategy. A first detection of climate warming? No, they would not go so far. But the work serves to illustrate many of the complexities associated with this issue.

The aim of the fingerprint approach is to detect the greenhouse effect as early as possible by proving that a specific aspect of expected anthropogenic greenhouse-induced climate change has already occurred within the climate record. It isn't a new concept^{2,3}, but its application has been hampered by a lack of adequate climate observations and model projections, the right mix of climate modellers and data analysts, and formal programmes focusing on climate change detection and attribution.

The fingerprint method applied by Karoly *et al.* entails testing for differences between observed and modelled fields of vertical temperature change. Rather than look at the longer records of surface temperature, they chose to focus on a warming of the troposphere (enhanced in the tropical upper troposphere) and a cooling of the stratosphere. Statistical tests indicate that the pattern of temperature change that has evolved in the observed data (recorded between 1963

and 1988) resembles the differences derived from control and doubled CO₂ simulations based on three different simulations from prominent general circulation climate models. The match is close enough for the odds to be roughly 9 to 1 against such congruence arising by chance alone.

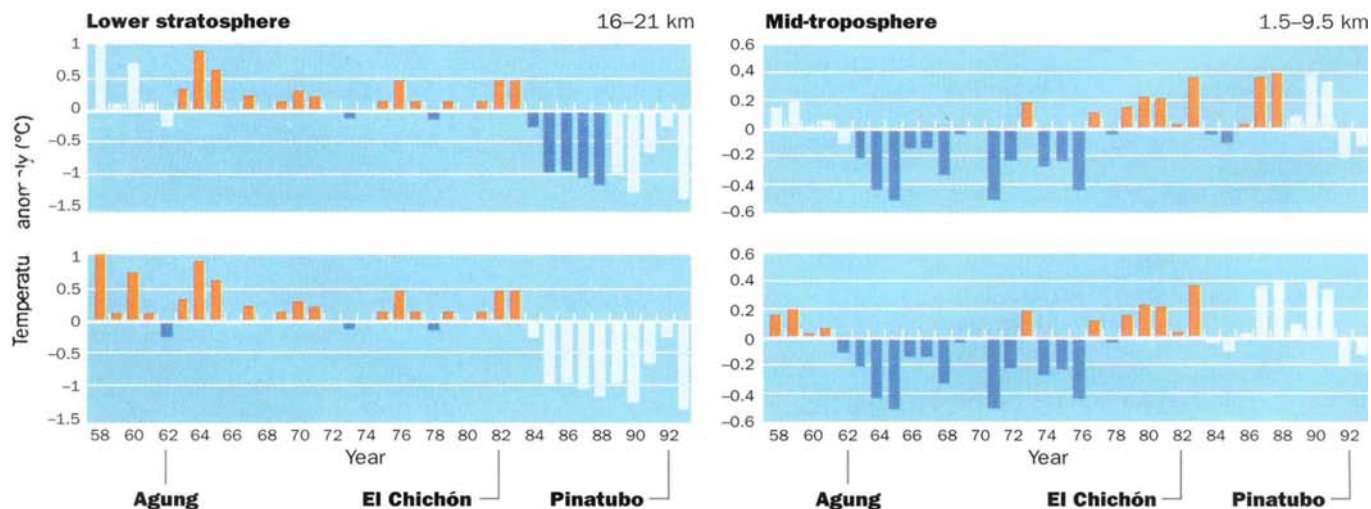
And now the complexities crowd in. Are the odds cited realistic and believable? Karoly *et al.* recognize that there are several other climate forcing mechanisms, besides the greenhouse effect, that have a direct bearing on the interpretation of their results. In particular, the depletion of stratospheric ozone has certainly contributed to stratospheric cooling; more frequent and stronger El Niño/Southern Oscillation events during the 1980s have contributed to a warmer troposphere; and if the global increase of sea surface temperatures during the 1980s, compared with earlier decades, is due to natural changes associated with deep ocean circulation, this non-greenhouse forcing would have also contributed to the observed tropospheric warming. Karoly *et al.* show that, individually, none of these mechanisms can explain the observed temperature changes, but obviously each contributes to the observed signal and inflates the odds.

Other potential problems, all of which Karoly *et al.* discuss, include: a short upper air record; observed data that are known to have time varying biases, especially in the high troposphere and stratosphere; a station network too sparse to resolve adequately the temperature changes in the high latitudes of the South-

ern Hemisphere; and the use of low resolution mixed-layer climate models without time-evolving greenhouse forcing.

The problem of short record length can readily be seen from the figure, adapted from a data set by Angell⁴. If Karoly *et al.* had selected data during the 25-year period from 1958 to 1984, a time when CO₂ concentrations increased at nearly the same rate as the period 1963–88, they would have found much less cooling of the stratosphere and little if any warming would be evident in the troposphere. This problem originates because of inadequate information about low-frequency natural climate variability of vertical temperatures — our present near-global coverage of observed data dates back only about 35 years. So model simulations are required to estimate the low-frequency natural variability, and Santer *et al.*⁵ have found that in transient simulations of an ocean-atmospheric general circulation model, the vertical structure of zonally averaged temperature change can mimic the greenhouse fingerprint in the limited data of the mixed-layer models used by Karoly and co-workers.

To my mind, there is no smoking gun to be found in the greenhouse detection (or rejection) issue. It would be very unlikely for any single application of a fingerprint strategy to provide adequate information about the impact of the anthropogenic greenhouse forcing in the climate record. This is simply because it is not possible to make use of all relevant observations and model simulations, yet the entire climate system is of interest, including expected changes in the hydrological cycle, atmospheric and ocean circulation, extreme weather events and climate variability. Unfortunately, the greenhouse signal-to-noise ratio for each variable differs⁵, and



The hazards of trying to detect a 'fingerprint' of climate change. These are annual anomalies of zonally averaged temperature based on the data set of Angell⁴. The top panel of each set shows the years analysed by Karoly *et al.*¹, indicated by the coloured lines and, as they note,

seems to show warming of the troposphere and cooling of the stratosphere. But shift the time interval sampled by a few years (bottom panels), and one sees little tropospheric warming and much less stratospheric cooling, despite similar greenhouse forcing.

this limits the practical importance of unequivocal detection of a greenhouse fingerprint in the climate record.

The implication is that even if Karoly *et al.* had claimed unequivocal detection of a greenhouse fingerprint we still could not claim confirmation of such a fingerprint in the ensemble of factors that constitute climate. This includes changes of surface temperature and precipitation⁶.

For the immediate future it is likely that we will have to settle for probabilistic statements about the likelihood of various aspects of climate change as greenhouse gases increase. What can we do to improve our confidence? First, we should brush up our ability to discriminate between anthropogenic forcing due to greenhouse gases and aerosols^{7,8}, and other forcings, especially those that may mimic a greenhouse fingerprint. This will require considerable investment in the diagnostics of model simulations, and must include identification of the most robust aspects of projected greenhouse-induced change. We must be able to assimilate into model simulations all of the potentially important time-varying forcings, such as stratospheric ozone loss, not just ignore them or dismiss them outright.

Second, we need reliable estimates of natural variability. High priorities must be given to long model simulations and the continued measurement and development of long instrumental and proxy data, free of serious time-varying biases. Currently, there are flaws in our ability to measure homogeneous long-term climate elements^{9,10}.

Finally, a critical linkage must be established between those variables affected by greenhouse-induced change and the concomitant biophysical and socioeconomic environmental variables that most strongly affect life on the planet. We must understand that detecting, for example, a thermometric greenhouse fingerprint in the climate record may mean little if it is not backed up by understanding of concomitant changes in other key aspects of climate. □

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The uncertain origin of introns

Laurence D. Hurst

AT first sight introns and sex have little in common, one point apart — for the evolutionary minded, it is not obvious why any organism should have either. If the textbooks¹ are to be believed, however, introns are ancient genetic devices that permit exons to be shuffled, so creating new and better proteins. This orthodoxy, the so-called exon theory of genes, is now under challenge, as became plain at this year's meeting of the Canadian Institute for Advanced Research's Program in Evolutionary Biology*.

At the core of the exon theory and its sometime alias, the introns-early hypothesis, are two principles (W. F. Doolittle, Dalhousie Univ.): that spliceosomal introns pre-date the divergence of bacteria and eukaryotes, and that modern gene structure provides some evidence for this; in other words, the exons that are shuffled may each encode some 'functional unit' of proteins. By contrast, the introns-late hypothesis has it that introns can be gained relatively easily, and that the positions that they currently hold in genes need not be because they have always been there. This hypothesis can also suppose that spliceosomal introns do not define 'functional units' but could, at an extreme, occur at random positions within the open reading frame.

Tests of the exon theory could hence be couched in the form of two questions. First, do introns divide genes into sequences that code for 'functional units'? Second, is the pattern of intron distribution across taxa consistent with an ancient origin?

Supporting the exon theory is the point that, for triose phosphate isomerase (TPI), the actual relationship between exons and protein structure is very different from what one would expect were introns randomly distributed (W. Gilbert, Harvard Univ.; ref. 2). The tests concerned were not explicit about what a functional unit is, but examined four properties such units might have (for example, a compact modular structure). Related tests, too, suggest that in TPI there might be a relationship between exon/intron arrangement and protein structure (A. Stoltzfus, Dalhousie Univ.; ref. 3). At the meeting Gilbert and Stoltzfus agreed that the exons of TPI have a significant tendency to code for compactly folded peptides. TPI may however be the exception, as three other proteins do not conform to this pattern (Stoltzfus; ref. 3).

Stoltzfus and colleagues have also sought evidence for correlations between

exon/intron structure and other aspects of protein topology. Protein biology has long understood that proteins contain α -helices and β -pleated sheets. If these are functional units then introns should be found between and not within sequences coding for them. Although introns in TPI exhibit a measurable tendency to fall between or at the edges of secondary structural elements (Stoltzfus), this tendency is not statistically significant. For the three other genes analysed, there is no significant tendency for introns to demarcate classical secondary structures³.

Is all this evidence against the exon theory? Certainly, it weakens the extreme position that introns always reside between sequences coding for α -helices and β -pleated sheets. Otherwise, it remains unclear whether the number of genes consistent with a nonrandom pattern is more than would be expected by chance. There are three possible problems (Gilbert). First, α -helices and β -pleated sheets are not the functional units that some of the introns-early models consider: what a functional unit might be will remain elusive until we have a better knowledge of the way proteins function. Thus Gilbert considers that the sort of test that Stoltzfus has performed is premature. Second, Gilbert argues that unless all of the putatively ancient intron positions are known, the tests performed by Stoltzfus could give a misleading result. Stoltzfus reported, however, that his methods can recover a signal, even when a substantial proportion of the data is missing.

Third, the tests assume that introns can either be lost or stay in the same position. Introns might however slide (change their position within a gene by means other than transposition). Gilbert reviewed evidence that strongly indicates that this is the case. Stoltzfus, in reply, showed that if slide is limited the signal can still emerge through the noise. But given long enough, slide could surely move introns over considerable distances. Even if we knew what a functional unit was, if sliding is put into tests of intron position the exon theory of genes would surely collapse into an unfalsifiable hypothesis.

Gilbert *et al.*⁴ did, however, predict that an organism would be found with an intron in a particular position in TPI. This position defined the middle of a bi-lobed protein structure. Although we do not know just what a functional unit is, this bi-lobed structure, Gilbert argued, is not one (more probably two) and hence should have an intron in the appropriate position.

This intron has been found in the mosquito *Culex*⁵. But has it been there since

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*Eighth Annual Meeting of the Program in Evolutionary Biology, CIAR, Dalhousie University, Halifax, Nova Scotia, 10–14 August 1994.

the earliest prokaryotes as introns-early requires, or did it recently move to this site? In a vigorous advocacy of introns-late, J. Palmer (Indiana Univ.) discussed the unpublished work of V. Walker and M. Tyshenko (Queen's Univ., Ontario) which, he argued, suggests a recent ancestry. Other than *Culex* and its close relative *Aedes*, Walker and Tyshenko have found no species with an intron in the 'Gilbert position', not even in other mosquitoes outside the *Aedes/Culex* subfamily. Surely, Palmer argues, a phylogenetic tree in which all but one group does not have a character is most parsimoniously interpreted as evidence of gain in that one, rather than losses in all other taxa. With an absence of spliceosomal introns in all prokaryotes⁶ the same logic can be more generally applied. The finding of rare intron positions simply adds to the number of losses (or slides) that must be invoked.

Although this argument may make gain more parsimonious than loss, if introns are only ever lost, every intron position in every gene must at some point be found in one species or group only. So introns-early is not incompatible with instances of unique intron positions. The above mode of analysis cannot then disprove introns-early, it just renders it unparsimonious.

Work by Palmer himself and his colleague J. Logsdon also counts against introns-early. The two have established⁶ that across eukaryotes there is a great deal of variation in the average number of spliceosomal introns per unit kilobase of coding DNA (from none to six introns per kilobase). They have now gone on to examine the titre of spliceosomal introns in genes that had been acquired from organelles but that currently reside in the nucleus. They find a good correspondence between the average intron number per kilobase of coding DNA for nuclear-located, organelle-derived, genes and the average for genes in the same organism that are ancestrally of nuclear origin.

This provides strong evidence that intron density, at least of the spliceosomal introns of nuclear genes, is dependent upon some feature of the organism that contains them. It is compatible with the introns-late model, as it assumes that introns can come and go at an appreciable rate, but problematic for introns-early. Spliceosomal introns have yet to be found in the putative sister groups of both mitochondria (alpha-purple bacteria) and chloroplasts (cyanobacteria). So to maintain that the positions of introns in the mitochondrial- and chloroplast-derived genes are ancestral, one has to argue that cyanobacteria and alpha-purple bacteria both had high titres of introns but subsequently lost them. This again is not impossible — just unparsimonious.

The new comparative data also provide the basis for tackling a different question:

what aspects of organismic biology can explain the variation in intron density? At least three sets of models attempt to account for this variation. First, selection in favour of an increased replication rate could select for reduced intron density. Second, introns may regulate the expression of genes involved in complex developmental strategies and their titre should hence be dependent upon developmental complexity⁷.

Third, sexual reproduction could provide the conditions for selection favouring increased intron density, for one of two reasons. If introns are selfish DNA they may spread in a population even if deleterious, but only if outbreeding events are regular enough⁸. Alternatively, introns may be a defence against the deleterious consequences of recombination. What if, as may indeed be the case, crossing-over causes flanking mutations (ref. 9 but see also 10)? Recombination within a gene could be deleterious but recombination in an intron would not. Alternatively, recombination may tend to cause chromosomal rearrangements if it occurs at illegitimate sites. Introns could tend to break up homologous sequences that occur at chromosomally non-homologous sites so preventing mispairing¹¹.

The three classes of models interpret the data differently. All can cite as support both the absence of spliceosomal introns in prokaryotes and organelles, as well as the low densities in unicellular eukaryotes. The replication rate model need simply invoke the idea that the above are all *r*-selected, the sex/recombination models need point to the fact that they are mostly clonal or with only occasional sex and the developmental regulation model need point to the developmental simplicity co-ordinated by the genomes concerned.

Through inter- and intra-genomic comparative analyses, we may be able to tease the forces apart. We may then find out what, if anything, introns and sex have in common, and just how unreasonable it is, given the absence of spliceosomal introns in prokaryotes, to suppose that ancestral bacteria were ever intron rich. □

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DAEDALUS

Blaze of colour

LAST week Daedalus suggested that a monolayer of oxygen adsorbed on a combustible surface could be explosive. If the top molecular layer of the surface could be made to burn in the adsorbed oxygen, the resulting two-dimensional explosion would propagate over the surface by Rayleigh surface waves. It could be used for cleaning and for controlled surface erosion.

He is now scaling up the idea. Conventional explosives need enough energy-density to propagate a blast in three dimensions. An explosive too feeble to explode in bulk might still manage to do so along a surface. One candidate is under-nitrated cellulose, used in many lacquers and varnishes. With the aid of special shaped-charge detonators designed to explode sideways, Daedalus is trying to explode the nitrocellulose varnish on old sideboards, television sets, filing cabinets, cars and so on.

Painters and decorators will await the results with keen interest. The most tedious part of their job is removing the old paint. A nitrocellulose paint could be blown off the wall by direct sideways detonation. Other paints might need to be nitrated first, by brushing on a special chemical paste. Better than any scraper and blowtorch, the explosion would propagate into all the nooks and crannies of the surface, leaving it cleaned and flamed ready for the new paint. This small, distributed explosion should do little structural damage. Its energy would be spread over the whole surface, and its shock would run parallel to that surface, rather than down onto it.

Exploding paint will be quite safe on the wall. Even in a fire it will burn quietly; only the special sideways detonator will make it explode. Every householder will adopt it, looking ahead to the time when it has to be replaced. Ship owners will welcome it — at present many ships are scrapped to avoid the cost of repainting. It will replace the waxes and transport coatings that protect cars during shipment. Once the car reaches the showroom, the retailer will blast the coating off again. The sudden surface heating may even briefly melt the conventional paint underneath, enhancing its gloss. Unscrupulous car owners, however, may use exploding paint as a permanent finish. They will spray their vehicles with an added explodable top layer in some contrasting colour. After an accident or brush with the law, they will blast it off again — it could even be arranged to detonate automatically on impact. The car will change colour instantly, confusing the witnesses.

David Jones

Arctic 'greenhouse effect'

SIR — Kahl *et al.*¹ show that the autumn and winter seasons during the past 40 years over the high Arctic have been significantly colder at the sea-ice surface. Moreover, the strength of the surface temperature inversion has increased by 7.8 °C. The authors argue that these results contradict the warming trend predicted by general circulation models. But Walsh in *News and Views*² argued that, when these observations are taken in a broad context, the results are consistent with model simulations of increasing CO₂. Because warming is occurring over land and on the fringe of the Arctic Ocean, the models predict the largest response in ice margins, away from the central region where the data used by Kahl *et al.* were collected. Nevertheless, the important surface cooling and the significant strengthening of the lapse rate over the Arctic Ocean remains to be explained.

The greenhouse warming effect resulting from water vapour is one of the chief causes of the observed global temperature rise. But the surface of the Arctic would be cooled if the water vapour concentration was reduced. The energy balance of the Arctic is mainly provided by import of moist and mild maritime air. This air mass is transformed by radiative cooling to polar air over a period of about 2 weeks. The process depends on the dehydration rate of the air. If this rate increases then by the greenhouse effect the surface cools at a faster rate. On the other hand, a lower H₂O concentration in the air reduces locally the infrared cooling rate of the air above the surface. The net result is a colder surface and a warmer air temperature aloft. This implies a steeper vertical temperature gradient (see ref. 1).

Almost all natural aerosol particles in the Arctic are coated by anthropogenic sulphuric acid^{3,4}. We have shown that acidification of Arctic aerosol (Arctic haze) can result in acceleration of the air-mass dehydration cycle⁵. A detailed and explicit simulation of the aerosol-cloud-precipitation-radiation processes in the Arctic atmosphere shows that an increase in the acid-to-insoluble-particles ratio reduces the number of activated nuclei, accelerates their growth rate, and increases the precipitation flux of atmospheric water. This process has been proposed to enhance precipitation in cloud seeding⁶.

Observations of Arctic haze⁷ support the idea that aerosol containing sulphuric acid produces fewer ice nuclei and results in fewer ice crystals. Controlled by the cooling rate of the ambient air, the condensation rate of atmospheric water vapour on fewer ice nuclei yields larger ice crystals. This effect substantially increases the total water flux to the surface and the

dehydration rate of maritime air that is enriched by excess anthropogenic sulphate during the cold Arctic season.

The Arctic haze is an aged anthropogenic aerosol. The aerosol particles achieve long lifetimes through the minimum removal rate and large static stability of the Arctic air. This process enhances the lifetime of aerosols by reducing their activation rate. To some extent, the aerosol is self-sustained in the Arctic region, prolonging its cumulative effect on the regional climate.

This mechanism provides a possible explanation for the observed trend during the past 40 years in the high Arctic, where the concentration of acid haze has been increasing^{8,9} because of long-range transport from industrialized regions.

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Crab hydrostatic pressure sensors

SIR — Adult crabs show locomotor-related responses to pressure pulses and altered rhythmic behaviour in response to pressure cycles^{1,2}. Planktonic crustacea show orientation changes with thresholds between 5 and 25 millibar (mb)^{1,3}. However, no pressure sensor has been identified and the lack of any obvious, compressible gas-filled partition has been considered a problem in making a sensor work^{4,6}. We report here that sensory hair receptors from the balancing organ, or statocyst, of adult crabs respond to step changes in hydrostatic pressure, and propose a mechanism involving mechanoreceptor activation by the alteration of the interior volume of cuticular hairs.

Angular accelerations are sensed by crabs by means of thread hairs and free hook hairs that are displaced by inertial fluid movements in the orthogonal canals of the statocyst^{7–9}. Thread hairs are also affected by gravity; their sensitivity is greatly dependent on the resting position of the hair. Individual hairs are sensitive over different ranges⁷: typically, a thread

hair responds over a 12–25° displacement range with phasic or tonic activity and maximum sensitivity of about 15 Hz per degree displacement. Each hair is innervated by two bipolar neurons with opposite directional sensitivities⁷.

Tonic and phasic-tonic increases and decreases in firing frequency of statocyst thread hair receptors occurred following increasing and decreasing pressure steps of 0.22 to 1.1 bar above ambient (Fig. 1). Significant differences were found between mean spike densities for 40 s before pressure changes compared with spike densities after pressure changes in 50 of 156 mixed units analysed. It was necessary to leave preparations for 15–30 min following a step of pressure to obtain a second full response, and variations in responsiveness or different proportions of positive or negative-going responses made construction of an intensity function inappropriate. Statocyst hairs in crustacea are unusual in that a chitinous chorda links the hair base with the dendrites which are sheathed by scolopale cells⁹ (Fig. 2b, c). In the crayfish statolith the chorda inserts on a cuticular spine, the lingula, near the base of the hair. There is evidence that the chorda of the narrower (2–4 µm) thread hair passes up the shaft of the hair and may have a loose hydraulic coupling within the hair (Fig. 2; and P. Dunn, personal communication). Volume changes within the interior fluid, arising from hydrostatic pressure, will lead to displacements of the chorda like the plunger of a syringe. The high hydraulic resistance, caused by the small diameter of the hair and the small opening through which the chorda passes at the base, will also help couple chorda displacement to volume change. The long, thin, cylindrical shape of the hair will help translate volume changes into displacements.

Consider a simple model with chorda coupled to the hair at its midpoint (Fig. 2). A 1° displacement of a 2-µm-diameter hair will lead to a displacement of 0.0175 µm of the chorda, assuming no slippage or elasticity. In the simplest case the effect of pressure on the hair can be modelled in the absence of hoop stress. Consider also the change of volume of contents of a 400-µm-long by 2-µm diameter hair to a 1-bar pressure change. Assume the compressibility of the hair cell interior is similar to that of sea water (43.9×10^{-6} per bar and the cuticle is relatively incompressible)⁶. If the diameter stays the same, then the length change following compression by 1 bar is $43.9 \times 10^{-6} \times 400 \mu\text{m} = 0.0176 \mu\text{m}$.

Considering the similarity of the calculated pressure-evoked displacement of the chorda to that following an above-threshold response, it seems unnecessary to invoke a compressible gas-phase transducer^{4,6}. This application of Enright's differential compressibility idea,

acting through hydraulic coupling of chorda with hair is sufficient to explain the sensitivity of the thread hairs to pressure⁶.

The geometry of the proposed system is

adequate to account for the way pressure alters the hair receptor firing patterns during oscillation. For example, during angular displacement, the chordae of up-

per and lower thread hairs will be displaced in opposite directions relative to the neurons⁷. Pressure increase will cause chorda displacements away from the neurons in both groups of thread hairs. Some statocyst interneurons are known to integrate activity from these groups of thread hairs separately, and these could have a role in sensing changes in pressure as well as the equilibrium state¹⁰.

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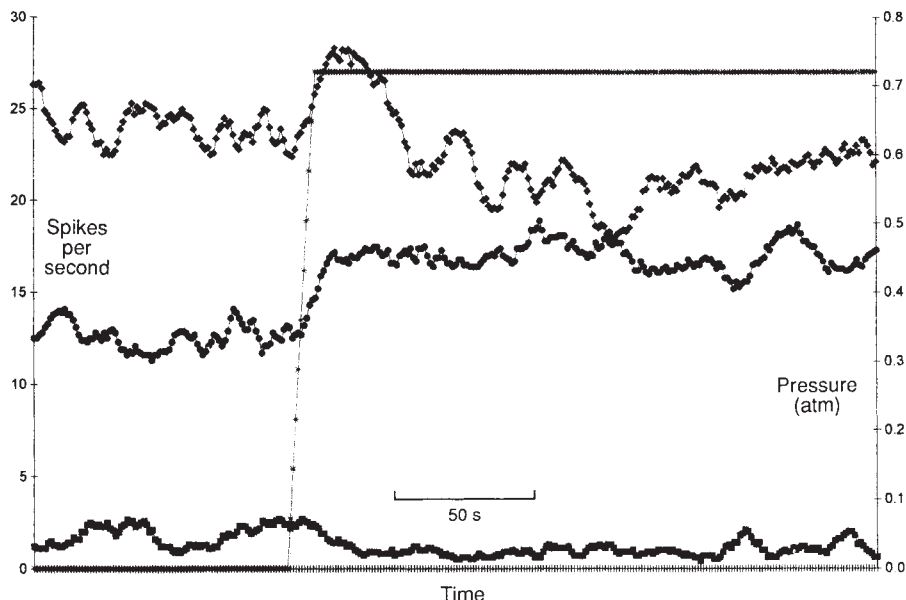


FIG. 1 Thread hair spike frequencies from an isolated antennule of the crab *Carcinus maenas*. Counts per second from three successive spike height ranges of an extracellular recording and the hydrostatic pressure were smoothed by a 10 point running average (smoothed units 1, 2 and 3 and smoothed pressure). Recording showing tonic increase, tonic decrease and phasic increase followed by tonic decrease in resting activity following an applied step of pressure 0.72 atm above ambient. Pressure was applied using compressed nitrogen over liquid paraffin in a pressure chamber similar to that used in ref. 11. Top trace, smoothed units 3; middle trace, smoothed units 1; bottom trace, smoothed units 2; (*) smoothed pressure.

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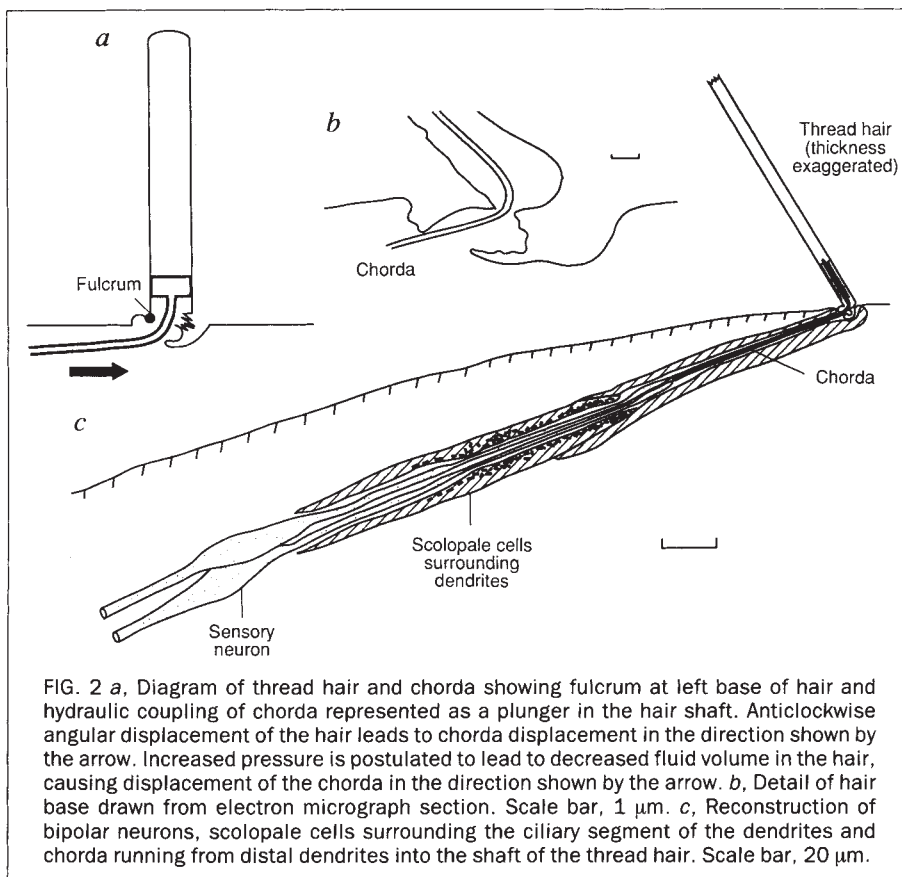


FIG. 2 a, Diagram of thread hair and chorda showing fulcrum at left base of hair and hydraulic coupling of chorda represented as a plunger in the hair shaft. Anticlockwise angular displacement of the hair leads to chorda displacement in the direction shown by the arrow. Increased pressure is postulated to lead to decreased fluid volume in the hair, causing displacement of the chorda in the direction shown by the arrow. b, Detail of hair base drawn from electron micrograph section. Scale bar, 1 μ m. c, Reconstruction of bipolar neurons, scolopale cells surrounding the ciliary segment of the dendrites and chorda running from distal dendrites into the shaft of the thread hair. Scale bar, 20 μ m.

Cheap fullerenes

SIR — The report by J. L. Atwood *et al.* (*Nature* **368**, 229–231; 1994) that C_{60} and C_{70} form inclusion complexes with calixarenes is a significant addition to our rapidly expanding knowledge of the complex chemistry of fullerenes. But the authors base their claim of the possibility of a 50-fold reduction in the cost of producing purified fullerenes on the retail prices quoted by Aldrich, \$2,800 and \$10,700 per gram for C_{60} and C_{70} , respectively.

Although Aldrich enjoys a reputation as a convenient supplier of high-quality fine chemicals for laboratory use, it is also one of the most expensive commercial sources of fullerenes. At the time when the paper by Atwood *et al.* was submitted for publication, fullerene manufacturers in the United States were offering purified C_{60} and C_{70} at retail prices of \$150–\$350 and \$1,250–\$3,500 per gram, respectively, in 1-gram quantities. As of March 1994 these prices had fallen to \$100–\$225 and \$600–\$3,000 per gram, respectively, with substantial discounts available for large orders.

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The Hubble constant and Virgo cluster distance from observations of Cepheid variables

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The distance to the Virgo cluster of galaxies, a primary rung on the ladder to establishing the distance scale of the Universe, 14.9 ± 1.2 Mpc, has been determined through ground-based observations of Cepheid variables in NGC4571. This agrees very well with other modern distance estimates, and the extragalactic distance scale now seems established. Based on this distance, a Hubble constant of $H_0 = 87 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1}$ has been calculated.

IMPORTANT and rapid progress is being made in establishing the extragalactic distance scale. The more modern and quantitative methods have now been defined and applied to a significant number of galaxies, so that an intercomparison and testing of these methods is now possible¹⁻³. The result is that these methods are all in good agreement, with the exception of type Ia supernovae (SN Ia) as calibrated by Sandage^{4,5}. However, SN Ia may not be as uniform as previously claimed⁶ and there may be notable problems in the photometry of the calibrating events⁷, such that the discrepancy may not be as significant as is often claimed. This discrepancy has led to the well known dispute about the absolute distance scale in the Universe, which is frequently characterized as a debate over the value of the Hubble constant.

It is now generally accepted that the discrepancy between the 'short' ($H_0 = 80\text{--}100 \text{ km s}^{-1} \text{ Mpc}^{-1}$) and 'long' ($H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) distance scales begins rather abruptly at a distance of about 7 Mpc (refs 3, 8). That is, the distances to nearby galaxies are in good absolute agreement and the distances beyond about 7 Mpc are in good relative agreement. Similarly, it can be shown that the various values for H_0 found in the literature correlate strongly with the reported distance for the Virgo cluster in those same sources. Thus, establishing the value of H_0 , to say 10%, can be done by establishing the distance to the Virgo cluster to a similar precision. This is the case even in the presence of significant large-scale peculiar velocities, as shown by the linearity of the Hubble diagram⁹ using either brightest cluster members¹⁰ or SN Ia (ref. 11).

The one method that has not been applied beyond 7.5 Mpc is also the one which holds the greatest promise in alleviating this discrepancy¹. This is the use of the Cepheid period-luminosity relation. A Cepheid is a pulsating variable star whose pulsation period is strongly correlated with its luminosity. By determining a Cepheid's period, its luminosity is immediately known, allowing its distance to be determined from its apparent brightness. These stars are well understood from both empirical and theoretical perspectives and have an important and well tested history of application¹². Consequently, it has been argued that the detection of Cepheid variables in the Virgo cluster is the best hope for establishing the extragalactic distance scale¹³. Historically,

this has been thought to be beyond the capabilities of ground-based telescopes and thus was used as a prime example to justify the need for space-based science. However, the continuing progress in ground-based, high-resolution imaging has made the prospects for such a survey much more feasible¹⁴.

We report here the results of such a survey, using the high-resolution camera (HRCam) on the Canada-France-Hawaii Telescope on Mauna Kea. We identified three probable Cepheid variables in an H I-depleted galaxy near the core of the Virgo cluster, and determined their periods. After correcting the apparent magnitudes of the Cepheids for extinction in the Milky Way along the line of sight to the Virgo cluster, we determined that the galaxy lies at a distance of 14.9 ± 1.2 Mpc. We have now established an absolute reference for the extragalactic distance scale. After correcting for the motion of the cluster with respect to the cosmic microwave background, we calculate a value for the Hubble constant, $H_0 = 87 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This high value, in conjunction with most currently fashionable cosmological models, conflicts with the ages of the oldest known stars.

Cepheid variables in NGC4571

NGC4571 was chosen as a promising target for a variable star survey given the expected performance of the HRCam on the Canada-France-Hawaii Telescope. It is a moderate-luminosity spiral (Sbc II; ref. 15) with a correspondingly lower disk surface brightness such that it should resolve relatively easily into stars¹⁶. In addition, it is sufficiently luminous to contain a reasonable number of luminous Cepheids¹⁴, of which we have isolated three. We first give arguments why we expect the distance of NGC4571 to be representative of the distance to the Virgo cluster.

NGC4571 is located less than 2.5° from M87 and shows signs of significant H I stripping³⁷. This provides strong evidence for NGC4571 being in the 'core' of the Virgo cluster, given the extensive evidence for the occurrence of stripping within the cores of rich clusters and the fact that the Virgo spirals with significant stripping are within 3° of M87 (ref. 38). Because the mean heliocentric velocity of the Virgo cluster ellipticals and lenticulars is $\langle v_\odot \rangle = 1,045 \pm 63 \text{ km s}^{-1}$ with a radial velocity dispersion of $\sigma = 556 \text{ km s}^{-1}$ (ref. 17), the low radial velocity of NGC4571 ($342 \pm 3 \text{ km s}^{-1}$) is further evidence for this galaxy being in or near the dynamically relaxed core of the cluster. The line-of-sight velocities for any particular galaxy projected upon an over-dense region are 'triple-valued' in the predicted distance

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using linear infall models of the cluster region¹⁸. However, when the velocities are low this ambiguity becomes minimal. For example, the Virgo-centric infall model of Tully and Shaya¹⁹ predicts distances for NGC4571 of 3.6, 15.5 and 19.1 Mpc, if the Virgo cluster is at 15.5 Mpc. Although this particular model predicts an infall velocity for the Galaxy of 300 km s⁻¹, the predicted distances are relatively insensitive to the infall velocity of the Galaxy. The first possible distance is easily eliminated because, if this were the case, the galaxy would be unique in both absolute size and luminosity for its luminosity class¹⁵ and would have been resolved into stars long ago. The second possibility is that the galaxy is in the core of the cluster, and the third is that NGC4571 is ~23% further away than the Virgo cluster 'core'. As we will soon demonstrate, the latter case will seem very unlikely given the distance we derive.

We acquired imaging observations of NGC4571 with HRCam at 13 epochs during the 1991, 1992 and 1993 seasons. HRCam is essentially a 'fast guider' which operates at frequencies of up to 200 Hz and is capable of correcting for the 'tip-tilt' component of atmospheric seeing by monitoring a nearby guide star. The characteristics of HRCam have been described in refs 20 and 21. A total of 75 usable images were acquired using the SAIC 1 and Loral 3 CCDs (charge-coupled devices) between 11 February 1991 and 27 April 1993. For each 'epoch', we typically took ~5, 15–20-min exposures in the R bandpass, shifting the image on the CCD by ~5 pixels between each to reduce the effects of cosmetic defects. The images were bias-subtracted and flat-fielded with a median of several twilight sky flats in the typical manner. The combined exposures of a given epoch produced an image with typical full-width half-maximum (FWHM) ~0.50 arcsec and a limiting magnitude of $R \sim 26$ mag. We also acquired 2 epochs in the V bandpass in order to measure $V-R$ colours for any variables we might find. The details of our observing procedures will be described in a separate paper (D.L.W. *et al.*, manuscript in preparation).

We obtained photometry of the resolved stars in NGC4571 via DAOPHOT/ALLFRAME^{22,23}. ALLFRAME was ideal for our survey because it performs simultaneous point-spread function fitted photometry on multiple image frames using a 'master' list of program stars with fixed relative position. This 'master' list was obtained by running DAOPHOT II on an image constructed from the median of the 53 best (FWHM ≤ 0.55 arcsec) individual exposures (Fig. 1).

The identification of variables proceeded as follows. First, a weighted mean magnitude, $\bar{R}$, was determined for each night for each star. Global mean V magnitudes were also determined and hence a mean $(V-R)$ index was formed. Using the mean $(V-R)$, the photometry was brought onto the same scale by correcting the photometry for colour terms. Only the first two epochs were found to have significant colour terms as these were the epochs for which a non-standard ' $V+R$ ' filter was used²⁴. Once the photometry was corrected for the colour term, a variability index²⁵ was formed using four sets of adjacent epochs. Three of these sets contained pairs of epochs and one set contained a triplet of epochs. Because the actual form of the variability index used is different from that in ref. 25, it will be briefly described.

For each star, for each epoch i , the number of detections, n , is established. From the set of magnitudes $R_{i,j}$ and uncertainties $\sigma_{i,j}$ available at each epoch, the j th residual is

$$\delta R_{i,j} = \frac{R_{i,j} - \bar{R}}{\sigma_{i,j}} \quad (1)$$

For that epoch, the contribution to the variability index, $\mathcal{J}_i$, was calculated to be

$$\mathcal{J}_i = \frac{2}{n_{j=1, n-1} \sum_{k=j, n}} \text{sign}(\delta R_{i,j} \delta R_{i,k}) \sqrt{\delta R_{i,j} \delta R_{i,k}} \quad (2)$$

where $\text{sign}(\dots)$ has an absolute value of one and the sign of

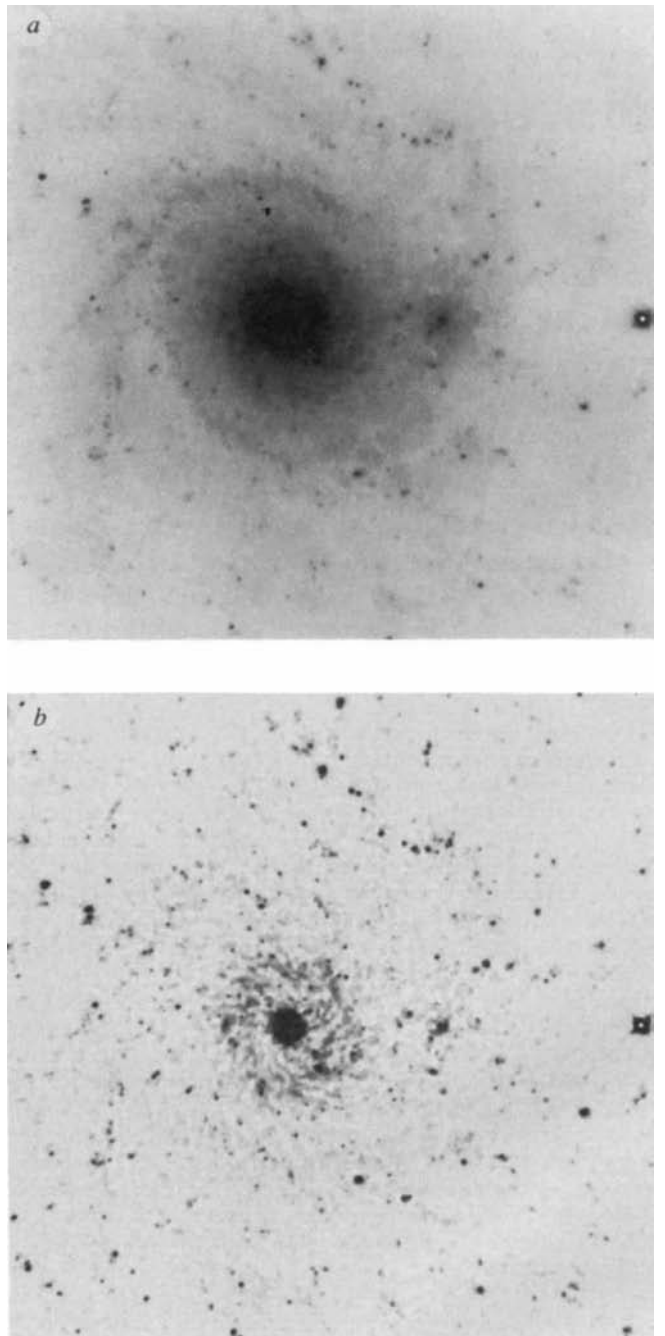


FIG. 1 a, A combination of 53 15–20-min exposures of NGC4571. The image has a FWHM = 0.49 arcsec and is ~150 arcsec on a side with 0.11 arcsec per pixel. b, The same image after a smoothed version of the residual image (with fitted stars subtracted) has been subtracted. The limiting magnitude of these images is $R \sim 28$. Note the numerous stars and star-forming regions.

the argument. The form of the product of the residuals is different from that in ref. 25 in that it reduces the impact of one epoch of correlated residuals on the final statistic. The final variability index, $\mathcal{J}$, for the N epochs with useful correlated magnitude residuals was then found by calculating

$$\mathcal{J} = \sqrt{\frac{1}{N(N-1)} \sum_{i=1, N} \mathcal{J}_i} \quad (3)$$

At this point, it was determined that a major source of false variable identifications was due to stars within a few pixels of

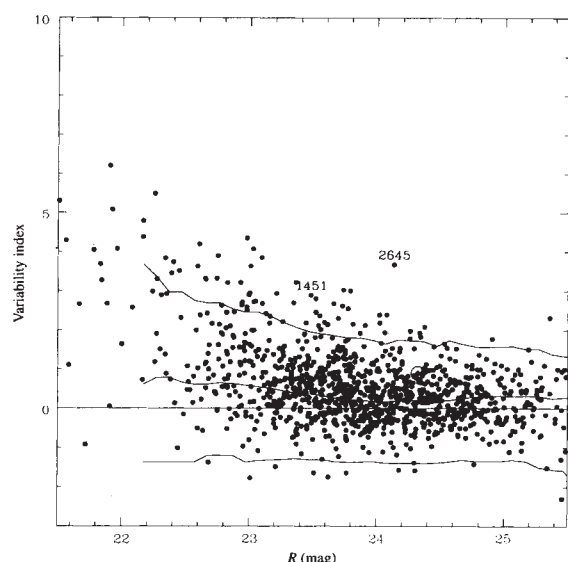


FIG. 2 The variability index²⁵ as a function of mean R magnitude. The three irregular curves indicate the 5th-, 50th- and 95th-percentiles of the variability index distribution as derived from the uncorrelated magnitude residuals of stars of similar brightness and crowding. Only stars further than 22 arcsec from the nucleus of NGC4571 were considered (see text). The three variables identified as Cepheids are indicated. Stars 1451 and 2645 were identified from their variability index, whereas star 2114 (the open circle within the general distribution) was identified from a visual examination of the photometry of the most isolated stars.

the CCD edge at more than one epoch, so potential variable candidates were checked for edge proximity on the original frames. Only stars with a full four sets of correlated epochs were considered further.

A plot of the variability index as a function of R magnitude appears in Fig. 2. In the absence of additional factors that might correlate magnitudes from different epochs (for example

systematic flat-fielding errors, close companions and so on), the distribution should be symmetrical about zero. Although this is not strictly the case, for the reasons outlined above, we can still assess the significance of variability for any particular star. Stars with unusually large positive variability index values are probably variable stars. Figure 2 also displays confidence intervals for the 5th-, 50th- and 95th-percentile points of the distribution found using correlations formed from the residuals of different stars within 0.5 magnitudes of the abscissa. Several candidates clearly warrant further inspection. Stars above the 95th-percentile were examined for spurious 'variability' due to correlated photometry with close companions, background nebulosity and so on. Two stars were judged as genuine variables with light-curves and periods (see below) consistent with Cepheids (1451 and 2645). In addition, we also did an independent visual examination of the photometry of all stars with $R \leq 25.0$ which were considered isolated (no companion within $\sim 2 \times$ the FWHM). Although subjective, this approach also identified star 2114 as a variable and produced one additional candidate Cepheid, 2114.

Calibrations were computed from a variety of standard stars (refs 26, 27 and L. Davis, personal communication) which showed agreement to within 2%. The ALLFRAME photometry was referenced to these calibrations using large-aperture photometry of the brightest, isolated stars in the images. The non-photometric nights were referenced to these nights through a robust mean zeropoint determination for each epoch (DAOMASTER).

A form of the 'string length' technique for finding likely periods, called the Lafler-Kinman technique²⁸, was then used to search for likely periods. We present the Lafler-Kinman statistic²⁸ (Θ), and best-fit lightcurve for our candidate variables in Fig. 3a-c. Although minima in the Lafler-Kinman statistic are associated with the true period, additional spurious minima resulting from chance phase alignments of points and aliasing also occur. The minima due to aliasing are generally present in each of the plots such that these could be identified. Most of the additional spurious minima do not result in reasonable light-curves when the data is phased with that trial period and can thus be eliminated as well. This procedure resulted in three stars

FIG. 3 The panels on the left show the Lafler-Kinman string length (Θ , see text) as a function of $\log(P)$ for each of the three candidate Cepheids. Note that aliases due to our limited number of epochs result in spurious features which generally repeat in each panel and can thus be eliminated from consideration. The adopted periods for each of the variables are indicated. The panels on the right illustrate the R -band light curves for the three candidate Cepheids with the periods given by the string length analysis. Each point is shown twice to allow continuity through zero phase to be assessed.

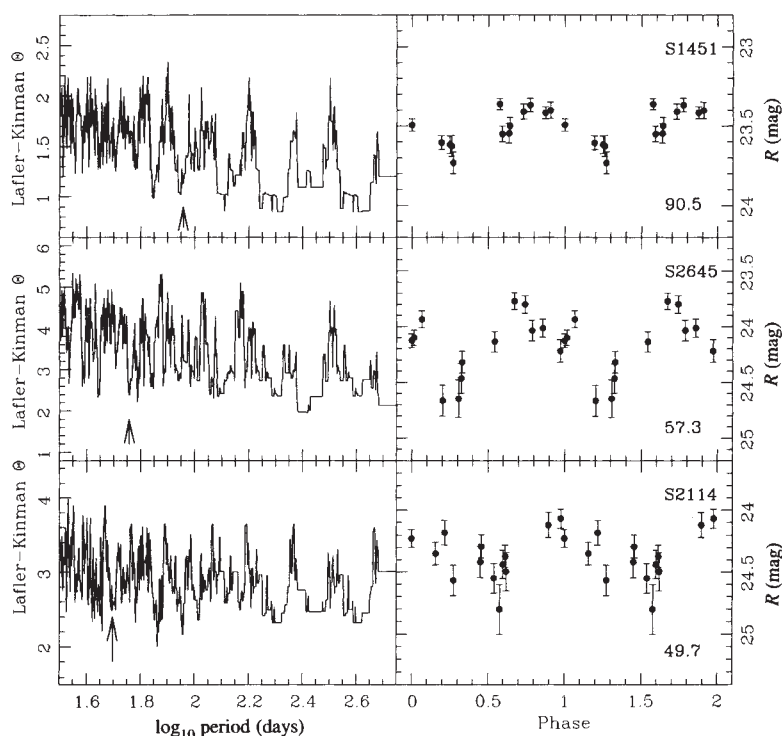


TABLE 1 Cepheid variables in NGC4571

Identification	$\log_{10} P$	$\langle R \rangle$ (mag)	$V-R^*$	$m-M^\dagger$
2114 [‡]	1.696	24.33	0.2	30.93
2645	1.759	24.14	0.5	30.93
1451	1.954	23.48	0.0	30.86

* We assign an uncertainty of ± 0.15 mag to the $V-R$ colours given the small number of epochs (two) for which we acquired V photometry.

[†] The apparent distance modulus (in mag) based on the LMC calibration¹² that assumes an LMC modulus of 18.5 mag.

[‡] A less certain variable selected from a visual examination of the photometry of the most isolated stars.

we concluded to be Cepheids (see Fig. 3). The photometry of these stars and their periods are given in Table 1, and are shown graphically in Fig. 4. We note that although the $V-R$ colours are uncertain they are sufficiently accurate to eliminate the possibility that these variables might be long-period variables, which have much redder colours ($V-R \approx 1.0$) than Cepheids ($V-R \approx 0.5$).

$P-L$ relationship and the distance to NGC4571

We now use the periods of the identified Cepheids to determine the distance of NGC4571. The period determines the absolute magnitude (luminosity) of each star, and we combine the results from all three to reduce the final uncertainty. After correcting the observed brightnesses for extinction from dust within the Milky Way, we calculate a distance of 14.9 ± 1.2 Mpc, which we take as the distance of the centre of the Virgo cluster.

It is apparent from Table 1 that only Cepheids over a relatively narrow range in period have been detected. Consequently, we followed the usual procedure of adopting the slope and zero-point of the $P-L$ relationship for Cepheids in the Large Magellanic Cloud (LMC) and computed the best-fitting relative distance modulus (Fig. 4). We adopt the calibration in ref. 12, assuming a distance modulus of 18.5 mag for the LMC; $M_R = -3.04[\log_{10}(P) - 1.0] - 4.48 \pm 0.08$ mag, where the period (P) is in days. The resulting apparent distance modulus for each variable was computed and is also given in Table 1. We find a mean apparent modulus for NGC4571 of $(m-M)_A = 30.91 \pm 0.15$ mag, where the uncertainty resulting from the finite width of the instability strip ($\sigma = 0.2$ mag; ref. 12) and that of the distance to the LMC contribute equally. This value must be corrected for any extinction by dust along the line-of-sight to the Cepheids in NGC4571. The extinction within our own Galaxy in the direction of NGC4571 is $A_B = 0.07$ mag (ref. 29), which

corresponds to $A(R) = 0.04$ mag, assuming a standard reddening law. Although there is likely to be some additional internal extinction within NGC4571 we cannot estimate this accurately given the limited precision in our colours for the Cepheids. However, the colour of the 'plume' brightest blue supergiants²⁴ suggests little reddening. Therefore, we assume no additional extinction for the Cepheids in NGC4571 and adopt $(m-M)_0 = 30.87 \pm 0.15$ mag. The corresponding distance is 14.9 ± 1.2 Mpc, assuming that NGC4571 is within the 3° core of the Virgo cluster.

We now consider two potential biases which might effect the distance we derived above. First, the effects of crowding will tend to bias the mean magnitudes too bright. That is a superimposed companion of similar brightness to the Cepheid will contaminate the photometry of the Cepheid. However, the presence of an undetected companion will also reduce the apparent amplitude of the Cepheid. For example, let us assume the Cepheids have an actual amplitude (in R) near the maximum found for those in the Large and Small Magellanic Cloud, with similar periods (0.3–0.7 mag; ref. 30). The observed amplitudes we find are 0.7, 0.5 and 0.3 for stars 2645, 2114 and 1451, respectively. Thus we can place limits of 0.0, 0.5 and 0.6 mag on the maximum contamination of these stars. The bluer colours of the latter two might be considered as evidence for contamination but the colours are rather uncertain. So, while we cannot rule out as much as 0.5 mag of contamination for two Cepheids, it must be pointed out that the luminosities of the contaminating stars would be extreme ($M(V) \approx -6.8$) and such stars are exceedingly rare. Furthermore, it would remain a mystery why we obtain the same distance for NGC4571 with these 'contaminated' Cepheids as we get using star 2645, which cannot be significantly contaminated. So, although we concede that contamination is possible we have no evidence of any contamination by close companions and we therefore make no correction to the estimated distance of NGC4571. A second potential bias has been argued to result from the finite width of the $P-L$ relationship coupled with a small range in sampled period. This is sometimes claimed to result in a selection bias such that the brightest variables at a given period will be preferentially detected in a magnitude limited survey. We only point out that the dispersion of the relation at R is only 0.25 mag (ref. 30) and any bias that might result is minimal.

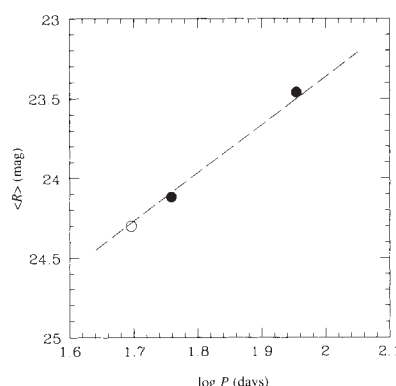
The Hubble constant

As argued above, the estimated distance for NGC4571 is very probably appropriate to the core of the Virgo cluster itself. In fact, this estimate agrees with the majority of other methods currently in use¹. Consequently, we consider this result a verification of these methods and it lends strong support to the view that the extragalactic distance scale is now established. Although one could argue that NGC4571 just happens to lie in the foreground of the Virgo cluster, such an argument seems extremely *ad hoc* given the evidence that NGC4571 is in or near the core of the cluster. The implications of such a 'short' distance scale are well known. Given that the relative distances between the Virgo cluster and more distant clusters are in good agreement we can compute the Hubble constant from the distance given above. We adopt a relative distance modulus between the Virgo and Coma clusters of $\Delta m = 3.71 \pm 0.05$ mag (refs 31–34) and a mean velocity for Coma of $\langle v_{\odot} \rangle = 6,888 \pm 86$ km s⁻¹ (ref. 35). With an additional correction of 258 ± 10 km s⁻¹ to place Coma in the reference frame of the CMB we find that

$$H_0 = 87 \pm 7 \text{ km s}^{-1} \text{ Mpc}^{-1} \quad (4)$$

This estimate assumes that no additional correction to the velocity of the Coma cluster is necessary because of peculiar motion. However, this value is likely to be representative over large scales given that there is no evidence for very large streaming motions out to scales of several hundred Mpc (refs 9, 10). The corresponding expansion ages for the Universe are

FIG. 4 The period–luminosity relationship for the Cepheids in NGC4571 and the mean relation for those in the LMC assuming $(m-M)_{\text{LMC}} = 18.5$ (dashed line). The solid points indicate those variables identified from the variability index (see Fig. 3 and text), and the open point indicates the additional variable (2114) found from an independent examination of the photometry of the most isolated stars. The adopted apparent distance modulus of $(m-M)_A = 30.91 \pm 0.15$ is derived from the mean of the three variables.



11.2 ± 0.9 Gyr and 7.3 ± 0.6 Gyr for $\Omega=0$ and 1, respectively. These values are well known to be in conflict with the estimated ages for metal-poor Galactic globular clusters (for example 16.5 ± 2 Gyr; ref. 36) and an inflationary cosmology with zero cosmological constant. The origin of this apparent paradox does not appear attributable to the observational data. $\square$

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Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire

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Two different approaches to follow clones of B lymphocytes in a diverse preimmune repertoire reveal a new process for eliminating self-reactive cells in the periphery which depends on competition between cells with different specificities. A key feature of this censoring mechanism is the selective exclusion of self-antigen-binding B cells from the normal migration route into lymphoid follicles, resulting in their premature death. This is a striking example of homeostasis by cellular competition for limiting niches and may explain the paradoxical association between immunodeficiency and autoimmunity.

BEFORE infection and exposure to new foreign antigens, a diverse preimmune repertoire of B lymphocytes continuously migrates between and through lymphoid tissues along defined routes¹⁻⁴. After entering secondary lymphoid organs, preimmune B cells migrate briefly through T-cell-rich zones and then move into discrete B-cell-rich primary follicles for 12–24 hours before exiting into lymph or blood and recirculating^{3,5-7}. In a healthy animal, the size of this B cell repertoire is tightly controlled, varying little from a mean of 2×10^8 cells in the mouse, the majority of cells being non-mitotic with a lifespan of more than four weeks^{5,8-11}. Only a fraction of the 10^{11} possible antibodies generated by immunoglobulin gene rearrangement in the bone marrow¹² are therefore displayed at any one time as surface immunoglobulin (sIg) receptors on recirculating B cells. How the size and composition of this long-lived recirculating repertoire is

controlled is of fundamental significance, because it is from this pool that antibody-forming cells are likely to be drawn immediately after infection. Moreover, deficits in follicular B cells accompany genetic and acquired immunodeficiencies¹³, whereas excess B-cell accumulation in follicles characterizes common human neoplasms such as follicular lymphoma^{14,15}.

To study the factors influencing entry and retention of particular B-cell clones in the preimmune repertoire, the frequency of B cells expressing a particular sIg specificity has been increased by engineering mice bearing rearranged immunoglobulin heavy chain (Hc) and light chain (Lc) transgenes¹⁶⁻¹⁸. This approach has confirmed that B cells that bind certain self-antigens undergo a cell-autonomous developmental arrest and are eliminated shortly after arising in the bone marrow¹⁹⁻²³. Other self-antigen-binding B cells recirculate between lymphoid follicles but cannot mount antibody responses owing to an intrinsic change (anergy) that desensitizes sIg signalling^{21,24-26}. The B-cell repertoire

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formed in Hc+Lc transgenic animals is, however, dominated by cells carrying the single antibody specificity defined by its rearranged transgenes; additional mechanisms responsible for shaping a normal, polyclonal repertoire might be identified if it were possible to add back clonal diversity. Here we have developed several approaches to follow antigen-specific B cells within a diverse preimmune repertoire, revealing a tolerance process that is not intrinsic to self-reactive cells but depends on competition with other B-cell clones. Competition preferentially excludes self-reactive B cells from entering follicles and results in cell death within three days. Death, but not exclusion from follicles, can be inhibited by constitutive expression of the follicular B-cell lymphoma proto-oncogene, *bcl-2*.

Heavy-chain transgenic mice

To create a model in which the fate of antigen-specific B-cell clones could be followed in a polyclonal preimmune repertoire, transgenic mice were produced carrying only the rearranged Hc gene encoding the heavy chain of an IgM and IgD antibody to hen-egg lysozyme (HEL)²⁴. Based on previous studies^{27,28}, pairing of the transgene-encoded Hc with endogenous light chains bearing V_k23 was expected to result in high-affinity HEL-binding IgM and IgD on a small fraction of B cells. Consistent with this prediction, 1% of the spleen B cells in Hc transgenic mice bound HEL as strongly as B cells in Hc+Lc transgenic mice, which are known to bind HEL with high affinity^{24,25} (compare window 1 in Fig. 1a and e). Additional subsets bound HEL less avidly (for example, window 2 in Fig. 1a) and similar frequencies of HEL-binding cells were observed in lymph nodes (not shown).

Our results showed that small numbers of HEL-specific B cells could contribute to a diverse preimmune B-cell repertoire in the absence of HEL antigen. To determine whether this was true for self-antigen-binding cells, Hc transgenic mice were analysed that carried an additional transgene which directed synthesis of HEL as a self protein in the bloodstream and extravascular fluid at different concentrations^{24,25,29}. Each of the strains of HEL transgenic mice show T-cell tolerance of HEL²⁹. In mice expressing HEL at low (10^{-10} M) concentrations (Fig. 1g), there was little change in low- or high-affinity HEL-binding B-cell numbers, consistent with previous findings that a threshold receptor occupancy is needed to trigger tolerance mechanisms^{25,29}. By contrast, the high-affinity HEL-binding B-cell subset became undetectable in spleen and lymph nodes of mice expressing 10^{-9} M HEL (Fig. 1b, window 1, and g), whereas lower-affinity HEL-binding B cells also disappeared in animals expressing 10^{-8} M HEL (Fig. 1c, window 2, and g).

Chimaeric mice

The disappearance of self-reactive B cells in the Hc transgenic animals, compared with persistence of self-reactive cells in the monoclonal repertoire of Hc+Lc transgenic animals (Fig. 1f for example), had two possible explanations: (1) the presence of other B cells altered the fate of self-reactive cells; or (2) there was an intrinsic difference in the recognition of HEL by cells in the Hc transgenic animals. To exclude the latter, bone marrow chimaeras were constructed with mixtures of Hc+Lc transgenic and non-transgenic bone marrow, thus adding back a polyclonal repertoire to otherwise monoclonal Hc+Lc animals. Other advantages of this approach were that Hc+Lc transgenic B cells could be distinguished independently of their HEL-binding IgM and IgD by use of allelic variants of the cell-surface marker Ly5, and the frequency of HEL-binding B cells and other non-binding B cells could be varied systematically. An optimal frequency of HEL-binding B cells, such that they were a minority but sufficiently numerous to be followed histologically, was obtained by reconstituting with a mixture of 80% Hc+Lc transgenic Ly5^b marrow cells and 20% non-transgenic Ly5^a marrow cells (80:20 chimaeric mice). These 80:20 chimaeras contained 20% Ly5^a bearing non-transgenic cells in all Ly5-positive non-B-cell haemopoietic lineages (not shown). Within the slg-negative pro-

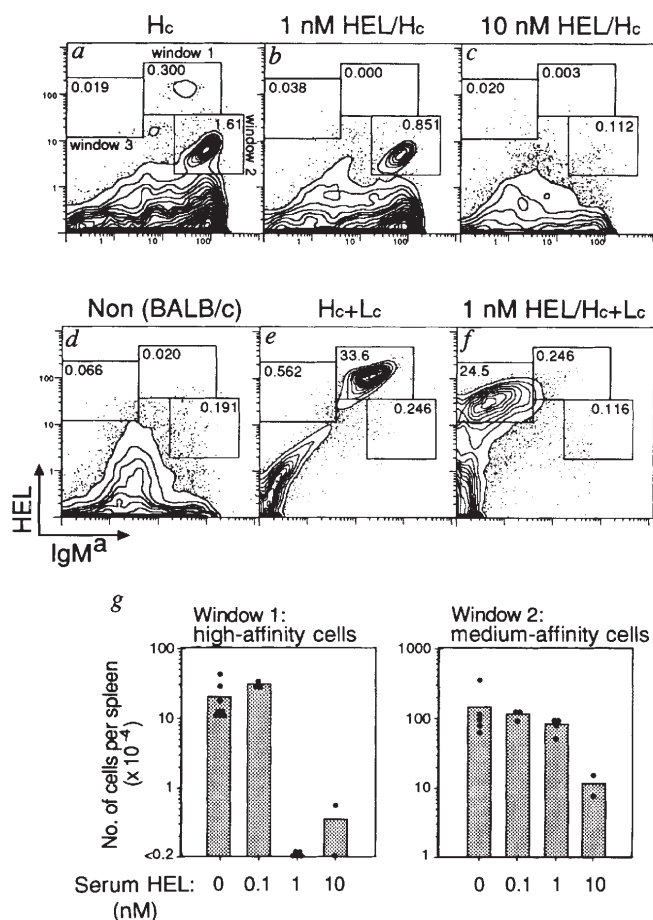


FIG. 1 Disappearance of autoreactive HEL-binding B-cell subpopulations from the polyclonal preimmune repertoire of Hc transgenic mice. a–f, Two-colour flow cytometric analysis for HEL-binding and the transgene-specific IgM^a allotype of spleen cells from the following mice: a, Hc transgenic; b, Hc and HEL transgenic with 1 nM serum HEL; c, Hc and HEL transgenic with 10 nM serum HEL; d, a BALB/c non-transgenic mouse as an IgM^a allotype control; e, Hc+Lc transgenic; f, Hc+Lc and HEL transgenic with 1 nM serum HEL. Two populations of HEL-binding cells are shown in a: cells binding HEL with high affinity (window 1) and cells with ~10-fold lower affinity (window 2). The percentage of splenocytes falling within each window is shown in a–f and absolute numbers from several animals lacking HEL or expressing 0.1, 1 or 10 nM serum HEL are plotted in g. Values from individual animals are indicated by dots and means by columns. In the monoclonal repertoire of HEL/Hc+Lc transgenic mice, high-affinity cells downregulate IgM^a, but not IgD^a (ref. 23); these cells are gated in window 3 (f). Note that the number of cells in window 3 is not above background in a–c. The mean number of total IgM^a-bearing B cells did not differ between the groups (0 nM HEL 25.2%, 0.1 nM HEL 30.8%, 1 nM HEL 27.5%, 10 nM HEL 34.2%).

METHODS. C57BL/6 (B6) strain MD2 Hc-transgenic mice and MD4 Hc+Lc transgenic mice³³ were either mated with B6-strain HEL transgenic mice or Ig-transgenic bone marrow was used to reconstitute the haemopoietic systems of lethally irradiated B6 strain HEL transgenic mice²². Equivalent results were obtained in both experiments. The HEL transgenic mice used^{25,29} were ML3 (0.1 nM), ML5 (1 nM) and AL3 (10 nM). Mice aged 6–14 weeks were used. HEL binding was measured by a sandwich immunofluorescence technique using 3×10^{-9} M unlabelled HEL followed by a biotinylated anti-HEL monoclonal antibody²⁴. Cell-surface IgM^a was detected with phycoerythrin conjugated RS3.1 antibody.

liferating pro-B-cell compartment, however, precursors from the non-transgenic inoculum expanded preferentially (not shown), presumably because rearranged immunoglobulin transgenes promote transition through the pro-B-cell stages with a reduced number of mitoses³⁰. As a result, 75% of the newly formed

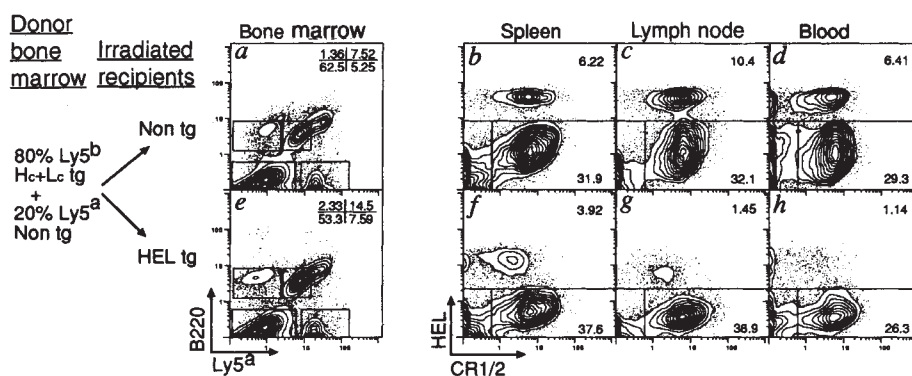


FIG. 2 B-cell competition excludes autoreactive B cells from the pre-immune repertoire in mixed bone-marrow chimaeras. *a, e*, Flow cytometry of bone marrow cells from a non-transgenic (*a*) or (1 nM) HEL-transgenic (*e*) mouse reconstituted with the indicated bone-marrow mixture, showing staining for Ly5^a and B220. B220^{lo} developing B-lineage cells are denoted in upper windows and B220-negative populations in lower windows. Note that not all B220-negative bone-marrow cells express Ly5. The percentage of cells in each window is shown. *b–d*, Peripheral lymphocytes from the chimaeric mouse in *a*, analysed for high-affinity HEL binding and the mature B-cell marker CR1/2. *f–h*, Peripheral lymphocytes from the chimaeric mouse in *e*, stained for HEL

binding and CR1/2. Percentages of HEL-binding B cells and of non-HEL-binding CR1/2-positive B cells are shown in the respective windows. **METHODS.** B6-strain-MD4 Hc+Lc transgenic mice³³ and B6-Ly5^a congenic mice (gift from I. L. Weissman) were used as donors. B6-strain-ML5 HEL-transgenic and non-transgenic X-irradiated recipients were reconstituted with $0.5\text{--}1 \times 10^7$ bone marrow cells per mouse and analysed after 4–6 weeks. Preparation of cell suspensions, immunofluorescent staining and flow cytometry were done as described²². When streptavidin-tricolour (Caltag) was used to detect CR1/2, a streptavidin-tricolour binding site on HyHEL10-HEL complexes was blocked by addition of unconjugated HyHEL9.

(B220^{lo}) bone marrow B cells were Ly5^a non-transgenic cells and only 25% were Ly5^b Hc+Lc transgenic cells (Fig. 2*a, e* and Table 1).

In chimaeras that lacked HEL antigen, the ratio of HEL-binding to non-HEL-binding B cells in the newly formed pool remained largely unchanged among mature B cells in the spleen, lymph nodes and peripheral blood (Fig. 2*a–d* and Table 1). By contrast, in chimaeras expressing HEL autoantigen no reduction in Hc+Lc transgenic B-cell numbers occurred in the newly formed bone marrow pool (Fig. 2*e* and Table 1) but the frequency and number of HEL-binding B cells was selectively diminished ~10-fold in lymph nodes and blood (Fig. 2*g, h* and Table 1). A less severe reduction in the frequency of HEL-binding cells occurred in the spleen (Fig. 2*f* and Table 1), possibly reflecting continual migration of newly produced B cells to this organ from the bone marrow³¹. This interpretation is supported by the lower expression of complement receptor 1/2 (CR1/2), a marker of B-cell maturity^{22,32}, on the HEL-binding autoreactive B cells present in the spleen (Fig. 2*f*). HEL-expressing bone marrow chimaeras reconstituted in parallel with 100% Hc+Lc transgenic bone marrow, where few other B cells

were present to compete against HEL-binding cells, showed little reduction in peripheral HEL-binding B-cell numbers (Table 1).

Exclusion of self-reactive B cells

The basis for repertoire skewing against autoreactive B cells in the mixed chimaeras was explored by immunohistochemical staining of lymphoid tissue sections (Fig. 3). In 80:20 chimaeric mice lacking HEL autoantigen, HEL-binding B cells were distributed evenly among non-HEL-binding B cells within the primary follicles and marginal zones of the spleen (Fig. 3*a*) and within primary follicles in lymph nodes (not shown). In mixed chimaera recipients expressing plasma HEL, however, few HEL-binding cells were present in follicles and the majority accumulated at the junction of the B- and T-cell zones (Fig. 3*b*) in the outer periarteriolar lymphatic sheath (PALS). A similar distribution was observed in lymph nodes, with HEL-binding cells restricted to the outer paracortical T cell zones and absent from follicles (not shown). Exclusion of HEL binding cells from follicles was not seen in HEL-expressing recipients reconstituted with 100% Hc+Lc transgenic bone marrow, where HEL-binding cells were

TABLE 1 B-cell competition excludes self-reactive B cells from the preimmune repertoire of mixed bone marrow chimaeras

Tissue: Recipient:	Bone marrow ($\times 10^{-5}$)*		Spleen ($\times 10^{-6}$)		Lymph node ($\times 10^{-5}$)		Blood (%)	
	Non-tg	HEL	Non-tg	HEL	Non-tg	HEL	Non-tg	HEL
100% chimaera	<i>n</i> = 9	<i>n</i> = 8	<i>n</i> = 9	<i>n</i> = 9	<i>n</i> = 9	<i>n</i> = 9	<i>n</i> = 4	<i>n</i> = 5
Total B cells†	11.8 (2.7)	14.6 (2.4)	29.6 (4.4)	30.2 (5.4)	31.0 (10.2)	29.3 (7.0)	30.6 (8.7)	19.6 (3.4)
Hc+Lc tg cells†	11.8 (2.7)	14.6 (2.4)	26.7 (4.2)	27.7 (5.4)	26.1 (10.5)	23.3 (7.0)	27.8 (7.4)	15.8 (4.0)
80:20% chimaera	<i>n</i> = 17	<i>n</i> = 17	<i>n</i> = 24	<i>n</i> = 24	<i>n</i> = 21	<i>n</i> = 21	<i>n</i> = 4	<i>n</i> = 5
Total B cells	21.1 (5.8)	24.0 (11.9)	52.4 (19.8)	52.1 (16.8)	48.4 (20.0)	49.3 (17.8)	42.2 (8.0)	35.5 (8.9)
Hc+Lc tg cells	4.4 (1.6)	6.2 (2.3)	9.6 (3.7)	3.9 (1.6)	9.7 (6.6)	1.3 (0.7)	9.5 (3.5)	1.5 (0.4)
	<i>P</i> < 0.02		<i>P</i> < 0.001		<i>P</i> < 0.002		<i>P</i> < 0.002	

Haemopoietic radiation chimaeras were generated as for Fig. 2 using 100% Hc+Lc transgenic bone marrow (100% chimaera) or 80% Hc+Lc and 20% non-transgenic bone marrow (80:20% chimaera) for reconstitution. Mice were analysed 4–9 weeks after reconstitution. No differences were observed after different periods of reconstitution.

* Bone marrow from one tibia and fibula.

† Total B cells represent all B220⁺ cells in peripheral tissues and all B220^{lo} cells in bone marrow. Hc+Lc transgenic (tg) cells represent B220⁺ HEL-binding cells in peripheral tissues and Ly5^a-negative, B220^{lo} cells in bone marrow. Data shown are arithmetic means with standard deviations in parentheses, and are from 5 experiments; *n* is the number of mice analysed for each tissue and group. Statistical comparisons are by the Student's *t*-test.

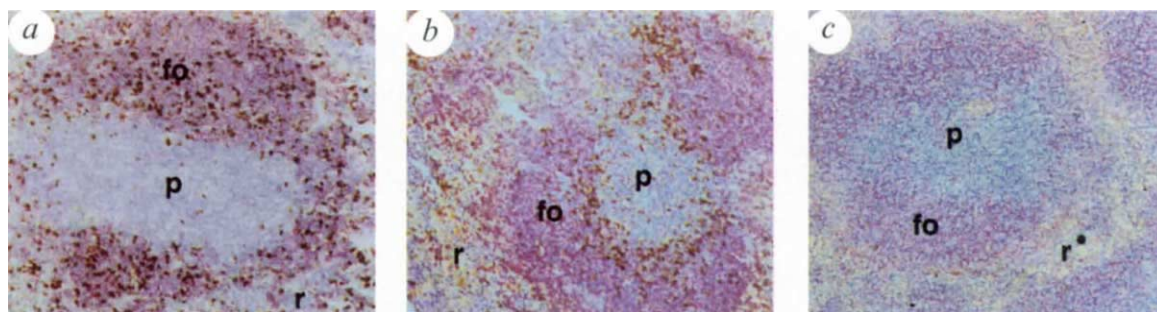


FIG. 3 Distribution of autoreactive and non-autoreactive HEL-binding B cells in spleens of mixed bone-marrow chimaeras. Two-colour immunohistochemical staining of spleen sections from mixed bone marrow chimaeras of the type used for Fig. 2, showing recipients lacking HEL (a) or expressing 1 nM HEL (b), and a non-transgenic mouse lacking HEL-binding cells (c). PALS (p), follicles (fo) and red pulp (r) are indicated. HEL-binding B cells are stained brown; all B cells show staining for B220 (red). In a, HEL-binding cells are distributed evenly among other

B cells in follicles, whereas in b, HEL-binding cells are concentrated in the outer PALS. A similar distribution was seen in spleens from ten chimaeras of each type and there was no change in cell distribution between 4 and 9 weeks of reconstitution. Magnification, $\times 40$. METHODS. Immunohistochemical analysis was done as described³³. B220 (RA3-6B2) staining was developed with anti-rat IgG-alkaline phosphatase-conjugated second antibody (Southern Biotech.).

evenly distributed throughout primary follicles (not shown), as previously found in HEL/Hc+Lc double-transgenic mice³³.

Elimination of excluded cells

Exclusion of autoreactive B cells from primary follicles in the mixed chimaeras indicated that the presence of other B-cell clones interfered either with entry of mature autoreactive cells into follicles or with a maturation step before follicular entry. To exclude the latter possibility and explore the fate of excluded cells, autoreactive B cells that had already matured and entered follicles in monoclonal HEL/Hc+Lc transgenic animals were transferred into HEL transgenic mice (HEL/Hc+Lc→HEL), where they would continue to bind HEL autoantigen but would now compete with a diverse repertoire of non-HEL-binding B cells. The fate of the transferred cells was compared with two control groups: (1) non-autoreactive B cells from Hc+Lc transgenic animals transferred into non-transgenic recipients (Hc+Lc→Non), where the transferred B cells would also compete with a polyclonal repertoire but would remain naive with respect to HEL autoantigen binding; (2) autoreactive cells from monoclonal HEL/Hc+Lc animals transferred into monoclonal

HEL/Hc+Lc transgenic recipients (HEL/Hc+Lc→HEL/Hc+Lc), where HEL autoantigen binding continued but the recipient repertoire consisted only of equivalent autoreactive B cells. The transferred cells were enumerated by flow cytometry and followed in tissue sections histochemically, using HEL binding, IgD^a allotype and Ly5^a to distinguish them from recipient B cells.

Six hours after transfer into animals with polyclonal repertoires, similar numbers of autoreactive B cells and non-autoreactive B cells reached the spleen (data not shown, and Fig. 6a). By 17 hours after transfer, however, the number of autoreactive HEL-binding B cells had decreased by 50%, and few autoreactive cells were detectable in spleen, lymph nodes or blood by 2.5 days (filled circles in Fig. 4a-c). By contrast, the number of transferred non-autoreactive cells was largely unchanged after 17 hours, and decreased only gradually over the next 4 days (open circles in Fig. 4a-c). Autoreactive B cells transferred into recipients that still carried HEL autoantigen but lacked a polyclonal repertoire (HEL/Hc+Lc→HEL/Hc+Lc; open triangles in Fig. 4a-c) persisted in a manner indistinguishable from transferred non-autoreactive B cells, indicating that

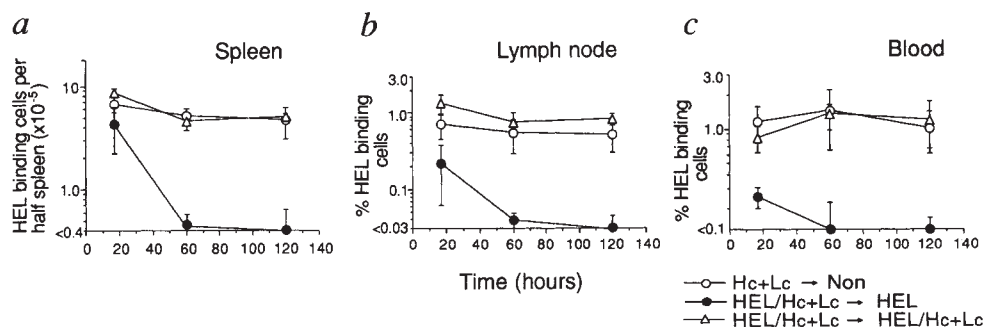


FIG. 4 Autoreactive HEL-binding B cells disappear rapidly from spleen (a), lymph node (b) and blood (c) after transfer to recipients with a diverse B-cell repertoire. Each point represents the mean of 3–5 recipient animals; error bars show standard deviation.

METHODS. Freshly isolated splenocytes from B6-strain transgenic donors were pooled, held on ice, and HEL-binding B cells counted by flow cytometry. Aliquots containing 1.3×10^7 HEL-binding or non-transgenic B cells in 0.3 ml medium were injected into the tail vein of unirradiated B6-strain mice. After 17 h, 2.5 days and 5 days, blood was taken, the spleen and mesenteric lymph nodes removed, and about half of each organ was frozen for sectioning. Cell suspensions were prepared from the remainder and analysed by flow cytometry. In

Hc+Lc→Non and HEL/Hc+Lc→HEL transfers, B220⁺, IgD^a positive, HEL-binding cells were counted. IgD^a was used as an additional marker as it is not downregulated much by HEL-binding²⁴ and so distinguishes transferred from endogenous cells. In some of these transfers, and all HEL/Hc+Lc→HEL/Hc+Lc transfers, Ly5^a donor cells were used; these were counted as Ly5^a positive, B220⁺, HEL-binding cells. Gating on Ly5^a positive, B220⁺ cells alone gave equivalent results, confirming that the HEL/Hc+Lc cells transferred to HEL recipients did not become undetectable as a result of receptor downregulation. The background level of events was determined using identical gates as for the HEL/Hc+Lc→HEL transfer with cells from non-transgenic mice that did not receive transferred cells.

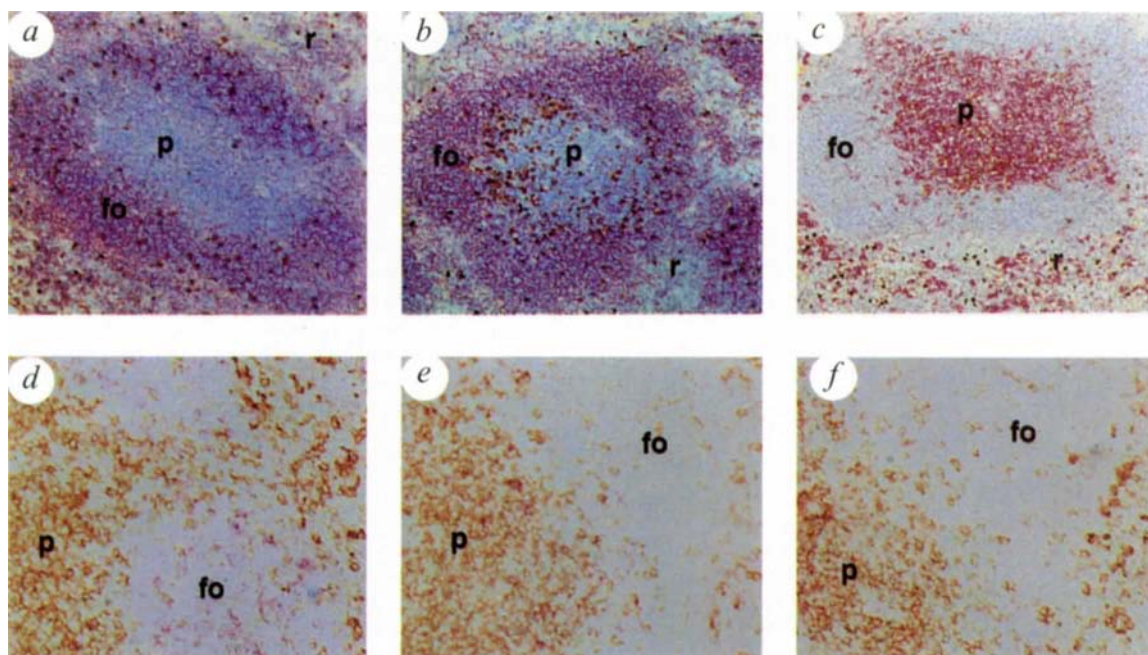


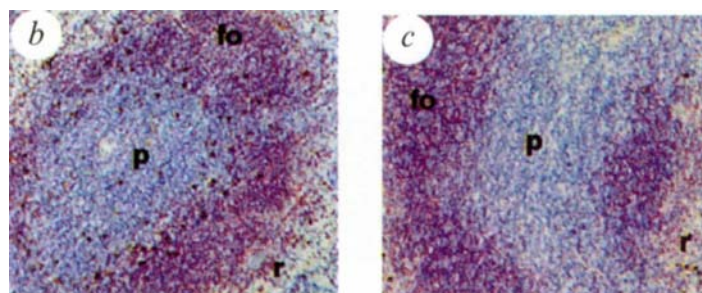
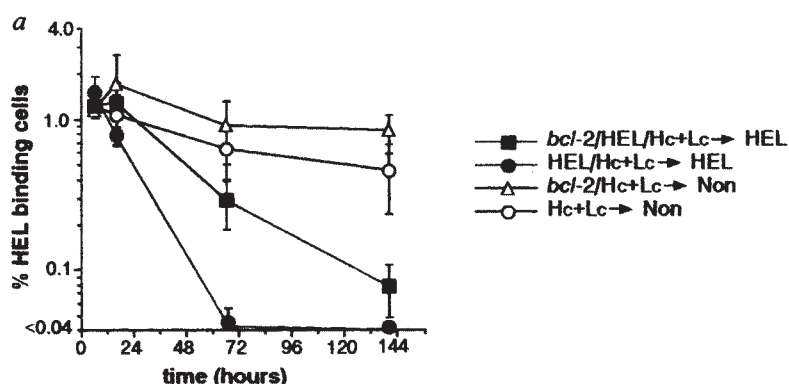
FIG. 5 Distribution of autoreactive HEL-binding B cells within the spleen 17 h after transfer to recipients with a diverse B-cell repertoire. *a*, Non-autoreactive Hc + Lc transgenic spleen cells in a non-transgenic recipient (Hc + Lc → Non), and *b*, autoreactive HEL/Hc + Lc cells in a HEL-transgenic recipient (HEL/Hc + Lc → HEL), stained for HEL-binding (brown) and B220 (red). *c*, Recipient spleen equivalent to *b*, stained for HEL binding (brown) and for CD4 and CD8 (red). Note intermingling of HEL-binding B cells with CD4⁺ and CD8⁺ T cells and paucity of HEL-binding cells in the follicles (fo). Results were similar from spleens of more than 15 mice of each type. *d–e*, Distribution of Ly5^a marked autoreactive HEL/Hc + Lc cells after transfer into *d*, an HEL/Hc + Lc

transgenic recipient (HEL/Hc + Lc → HEL/Hc + Lc), or *e*, a HEL-transgenic recipient (HEL/Hc + Lc → HEL). *f*, Control spleen from a recipient that did not receive Ly5^a-marked cells. Sections *d–f* are stained for Ly5^a-bearing cells (red) and for CD4⁺ and CD8⁺ cells (brown) and are not counterstained to allow better visualization of the Ly5^a-staining B cells. Note presence of cells stained red only (transferred B cells) in the follicles (fo) in *d* but not *e*. Magnification in *a–c*, ×40; *d–f*, ×80.

METHODS. CD4 and CD8 (Caltag) staining was developed with anti-rat IgG-alkaline phosphatase (c) or horseradish-peroxidase-conjugated (*d–f*) second antibody (Southern Biotech.). Ly5^a detection was with AS20-biotin followed by avidin-alkaline phosphatase.

FIG. 6 Autoreactive B cells expressing a *bcl-2* transgene show enhanced survival but continue to be excluded from follicles after transfer to recipients with a diverse B-cell repertoire. Splenocytes from donor mice carrying the indicated transgenes were transferred into HEL-transgenic or non-transgenic recipients as described for Fig. 4. *a*, HEL-binding, IgD⁺-positive, B220⁺ splenocytes were counted by flow cytometry 6 h, 18 h, 3 d and 6 d after cell transfer. The mean and standard deviation for 3–6 recipients at each time point is plotted. *b* and *c*, Immunohistochemical staining of spleen sections from HEL-transgenic recipients, three days after transfer of either *b*, autoreactive HEL-binding cells carrying the *bcl-2* transgene (*bcl-2*/HEL/Hc + Lc → HEL); or *c*, autoreactive cells lacking the *bcl-2* transgene (HEL/Hc + Lc → HEL). At this time HEL-binding (brown) cells can be detected in the outer PALS in recipients of *bcl-2* transgenic cells (*b*) but are undetectable in recipients of cells lacking the *bcl-2* transgene (*c*). Dark-staining cells in the red pulp are granulocytes and are seen in the absence of HEL staining.

METHODS. *bcl-2*/HEL/Hc + Lc and *bcl-2*/Hc + Lc transgenic mice were bred as described²² using *bcl-2-22* transgenic mice³⁴ that were a fourth generation backcross with C57BL/6J.



the rapid elimination of autoreactive cells in polyclonal recipients was not due to autoantigen binding *per se*.

Immunohistochemical analyses of spleen and lymph node sections from the recipients showed survival to be correlated with follicular entry. Non-autoreactive HEL-binding B cells (Hc+Lc→Non) entered and were evenly dispersed within follicles 6 h after transfer and their distribution changed little by 17 h (Fig. 5a) or 5 days (not shown). By contrast, autoreactive HEL-binding B cells in polyclonal recipients (HEL/Hc+Lc→HEL) were not present in follicles at 6 or 17 h but accumulated in the outer PALS of spleen (Fig. 5b and c) and in the outer paracortex of lymph nodes (not shown). After 17 h few or no autoreactive HEL-binding cells were detectable in sections. Autoreactive B cells followed with the Ly5^a marker were also not detected in follicles after transfer into polyclonal HEL-transgenic recipients (Fig. 5e) but were evenly distributed among primary follicles 6 or 19 h after transfer into monoclonal HEL/Hc+Lc transgenic recipients (Fig. 5d).

B cells expressing a *bcl-2* transgene

The rapid disappearance of autoreactive B cells excluded from follicles implied that the cells died *in situ*, although it was possible that they migrated elsewhere. To distinguish between these possibilities, programmed cell death was inhibited by constitutively expressing a *bcl-2* transgene³⁴ in the donor HEL/Hc+Lc B cells. Autoreactive B cells from monoclonal *bcl-2*/HEL/Hc+Lc triple-transgenic animals were transferred into polyclonal HEL-transgenic recipients (*bcl-2*/HEL/Hc+Lc→HEL) and their migration and survival compared with autoreactive cells lacking the *bcl-2* transgene (HEL/Hc+Lc→HEL) or with non-autoreactive B cells carrying or lacking *bcl-2*. Six hours after transfer, similar numbers of each transferred B-cell group had accumulated in the spleen (Fig. 6a). By 18 h autoreactive cells lacking *bcl-2* had decreased in number by 50% as before, but those expressing the *bcl-2* transgene remained unchanged (Fig. 6a). No autoreactive cells could be detected three days after transfer in HEL/Hc+Lc→HEL recipients by flow cytometry or immunohistochemistry (Fig. 6a and c), whereas autoreactive cells carrying the *bcl-2* transgene persisted at 30% of their original numbers at this time, and a small fraction could still be detected six days after transfer. Despite the enhanced survival of autoreactive B cells carrying the *bcl-2* transgene, they continued to be excluded from follicles and remained in the outer PALS three days after transfer (Fig. 6b).

Discussion

This study has revealed a novel process for eliminating self-reactive B cells and defines a cellular basis for this censoring mechanism. Elimination of self-reactive B cells as a result of competition with B cells bearing other sIg specificities was observed in three experimental systems: Hc-only transgenic mice, mixed bone-marrow chimaeras, and short-term transfer recipients. In each case, competitive elimination was dependent on binding a soluble self-antigen that is distributed throughout the body in extracellular fluids. The heavy-chain transgenic animals established that B-cell elimination was not a consequence of immunoglobulin transgene expression *per se*, as all B cells in these mice expressed a common heavy chain transgene but only cells that bound a threshold amount of self-antigen were outcompeted. Competition occurred at the level of entry into spleen and lymph node follicles in the bone marrow chimaeras and short-term transfer recipients, where the frequency of self-reactive B cells was sufficient to permit detection by immunohistochemistry. Furthermore short-term transfers allowed the kinetics of B-cell survival to be followed, demonstrating that B cells excluded from follicles have a half-life of less than one day, and that exclusion and elimination are not intrinsic to autoantigen-binding B cells. The effects of *bcl-2* showed that rapid elimination of self-reactive cells was due to death *in situ*, and that failure to enter follicles was not secondary

to death. Collectively, these observations establish a new role for follicular microenvironments as limiting niches that promote survival and retention of selected B cells, and identify a unique mechanism for maintaining self-tolerance.

It has long been accepted that B cells compete for a place in the recirculating repertoire^{2,5,11,35}. Precisely what recirculating B cells compete for has remained unclear, although studies of adoptive immune responses in irradiated animals led to the notion of competition for space^{36,37} and more recent transfer experiments have pointed to the possibility of competition for follicular entry³⁸. We establish here that competition between B cells of different specificity indeed takes place at the level of entry into the follicular microenvironment and that entry is accompanied by cell survival. These features resemble the cellular competition for restricted survival factors that is well characterized in the nervous system³⁹ and has been postulated to be a general mechanism for controlling both cell number and location⁴⁰. An analogous competition may occur between B cells for access to a trophic factor present in the follicular microenvironment.

Several observations suggest that the competitive exclusion process analysed here in transgenic animals plays a significant role in shaping normal B-cell repertoires. First, stereotypic shifts in the B-cell repertoire occur during development in man and mouse, causing V segments common in neonatal B cells to become infrequent in the mature steady-state repertoire^{9,41-43}. As some of these neonatal V regions tend to encode autoantibodies^{44,45}, their progressive disappearance could be explained by competitive exclusion from fully occupied follicular niches. Second, once at steady state, only a fraction of the B cells exported from the bone marrow each day enter the long-lived recirculating B-cell repertoire^{31,35}. Under these conditions, new B cells taken from the bone marrow are excluded from entering adult follicles in preference to B cells already in the recirculating pool³⁸. Although follicular exclusion could in this case reflect differences in B cell maturity, the striking similarity with the trapping of autoreactive B cells in the outer PALS observed here would be consistent with a high frequency of autoreactive cells in the less-selected immature pool^{44,45}. Finally, a general role for sIg engagement in preventing follicular entry is supported by the fact that non-autoreactive B cells that have bound foreign antigens during the initial phases of immune responses also accumulate transiently in the outer PALS^{11,46,47}. B cells binding foreign antigens may be rescued from death in the PALS by productive interaction with specific helper T cells, but rescue by this route is precluded for autoreactive cells because of the desensitization of sIg signalling in autoreactive B cells^{24,26} and the absence of self-specific helper T cells.

A key difference between competitive exclusion and other established mechanisms for maintaining self-tolerance such as deletion or anergy is that the latter represent cell-autonomous processes with an intrinsic triggering threshold¹⁸. By contrast, the efficiency of repertoire purging by competitive exclusion depends on the overall number and diversity of competing cells. This is an important distinction because it may explain the paradoxically high frequency of systemic autoimmune diseases such as autoimmune haemolytic anaemia in children with inherited deficiencies of recirculating B cells¹⁵. In these individuals, competitive exclusion of autoreactive B cells would be expected to be relaxed, whereas cell-autonomous tolerance processes should continue with equal efficiency. As the size of the recirculating preimmune T-cell repertoire also appears to be tightly controlled, it will be important to determine whether similar mechanisms account for the linkage between circulating T-cell deficiency and autoimmunity. □

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LETTERS TO NATURE

Collisional pumping of SiO masers in evolved stars

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ALTHOUGH SiO masers (lasing systems at microwave frequencies) have been detected in many old red-giant stars^{1,2}, several fundamental questions, such as what drives the masers and where in the stars' environment they are located³, remain unresolved. Evolved stars are known to have winds, which distribute enriched material throughout the interstellar medium, but the acceleration mechanism and the point in the stellar atmosphere at which the winds are initiated, are unknown⁴. SiO masers have high brightness temperatures, which allows them to be studied with high spatial and spectral resolution using very-long-baseline interferometry (VLBI) techniques, and thereby potentially determine how the stellar winds are generated. This will only be possible, however, once the physical conditions giving rise to the maser emission are known. Here we present images of positionally coincident maser emission from the $J=1 \rightarrow 0$ rotational transition of the first two vibrational levels in the SiO masers in VY Canis Majoris and W Hydrae. These results clearly demonstrate that the maser emission is collisionally pumped in distinct regions, rather than radiatively pumped. This means that SiO maser emission can be used to follow the clumps of gas as they are accelerated in the stellar atmosphere.

The simultaneous VLBI observations of two SiO maser lines ($J=1 \rightarrow 0$) in the vibrationally excited states $v=1$ (43.122 GHz) and $v=2$ (42.821 GHz) from VY Canis Majoris (VY CMa) and W Hydrae (W Hya) were made on 1 March 1991 with the Kashima–Nobeyama interferometer (KNIFE)⁵. The KNIFE consists of the Kashima 34-m radio telescope of the Communications Research Laboratory (CRL), and the Nobeyama 45-m

radio telescope of the National Astronomical Observatory, separated by ~197 km in an east–west direction, producing a minimum fringe spacing of 7 mas at 43 GHz. We observed respective sources for ~5 hours with the K-4 system⁶. Simultaneous observations of the two SiO lines simplified the visibility calibrations and made possible the reliable comparison of their spatial distributions, because gain and delay errors arising from atmosphere and instruments were equally cancelled and because they were free from monthly variations of SiO masers. Masers are generally distributed as clusters of emission spots. In our observations, most velocity channels ($\Delta v = 0.05 \text{ km s}^{-1}$) contained more than one maser spot. We therefore determined the positions and intensities of the SiO emission at each velocity channel with Fourier inversions and CLEAN algorithms⁷ with full loop-gain, assuming that the maser are distributed as clusters of emission points. The CLEAN subtractions were continued until the residual powers were below the 3σ level (30 Jy). Figure 1 shows one example of the visibility data. In order to estimate the positional errors of our mapping, arising from limited visibility data, we did CLEAN simulations using artificial data with a signal-to-noise ratio of 3 (where the thermal noise was generated by Monte Carlo methods). In the case of a single point in the image, the positional error was surprisingly small. The standard deviations were (right ascension) $\Delta\alpha = 0.007 \text{ mas}$ and (declination) $\Delta\delta = 0.208 \text{ mas}$. For two points in the image (the brightness ratio of the two points was 1:0.95), the standard deviations were $\Delta\alpha_1 = 1.17 \text{ mas}$ and $\Delta\delta_1 = 1.30 \text{ mas}$ for the first point, $\Delta\alpha_2 = 1.82 \text{ mas}$ and $\Delta\delta_2 = 1.49 \text{ mas}$ for the second point. The positional accuracy becomes worse as the number of points in the image increases. Fortunately the actual number of CLEAN subtractions or the number of spots was two or three at most in each velocity channel, and we estimate that the typical positional error is about 2 mas.

VY CMa is thought to be an M-type supergiant and W Hya is a semiregular variable. Both types belong to post main-sequence, evolved objects. Figure 2a, b shows the total intensity maps of the SiO $J=1 \rightarrow 0$, $v=1$ and $v=2$ lines for VY CMa. Figure 3a, b shows the same for W Hya. In each source, the $v=1$ and $v=2$ maser spots are spread over ~100 mas in angular size and have almost the same distribution. Infrared observations⁸ at 1.65 μm show that the angular diameter of the photosphere or, more probably, the inner dust shell of VY CMa is 133 mas (or 200 AU at a distance of 1.5 kpc). This is larger than the extent of the

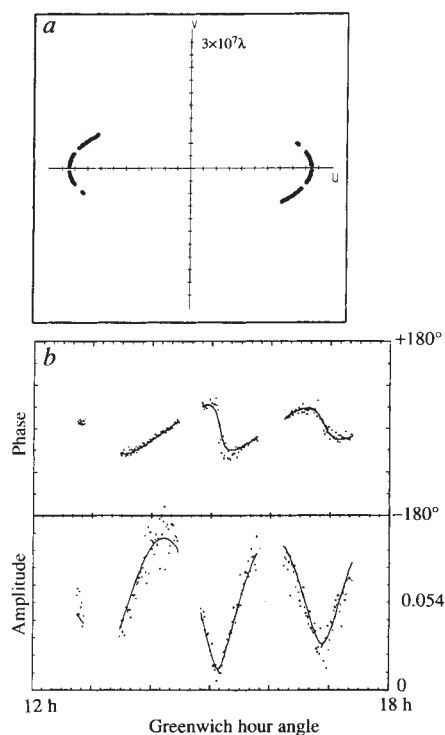


FIG. 1 Examples of visibility data from the observations of VY CMa. *a*, Actual (u, v) coverage; u and v are antenna spacing coordinates in units of wavelength (spatial frequencies). (Ten tick-marks on either axis correspond to 3×10^7 wavelengths.) *b*, Visibility data and the fitting curve derived from Fourier inversion of the CLEAN map for the SiO $J=1 \rightarrow 0$, $v=1$ emission at $v_{\text{LSR}} = 22.76 \text{ km s}^{-1}$ (v_{LSR} , velocity in local standard of rest). We used the K-4 system⁵ as a data recorder, which could record the data continuously for $>4 \text{ h}$ (in the 64 megabit per second recording mode with 16 channels of 2 MHz bandwidth). The total on-source time was 192 min for VY CMa, and 181 min for W Hya. The correlation processing was done with a new correlator (NAOCO)¹⁶ at Nobeyama in October 1992. The 512 point complex cross-correlations from the correlator were Fourier-transformed to cross-power spectra (with a velocity resolution of $\sim 0.05 \text{ km s}^{-1}$ at 43 GHz) and were coherently integrated in every 60 s. This resulted in 192 points of visibility data for VY CMa, and 181 points for W Hya. Visibility calibrations were done as follows. Residual delay offset which causes a phase slope was corrected by $0.5 \mu\text{s}$ (obtained from observations of continuum radio source 3C84). Amplitude fluctuations of each velocity channel caused by atmospheric and instrumental effects were removed by dividing by the amplitude of spatially unresolved velocity channels for both rotational lines ($v_{\text{LSR}} = 22.17 \text{ km s}^{-1}$ in VY CMa and 36.18 km s^{-1} in W Hya). These channels were also used as phase references for other velocity channels.

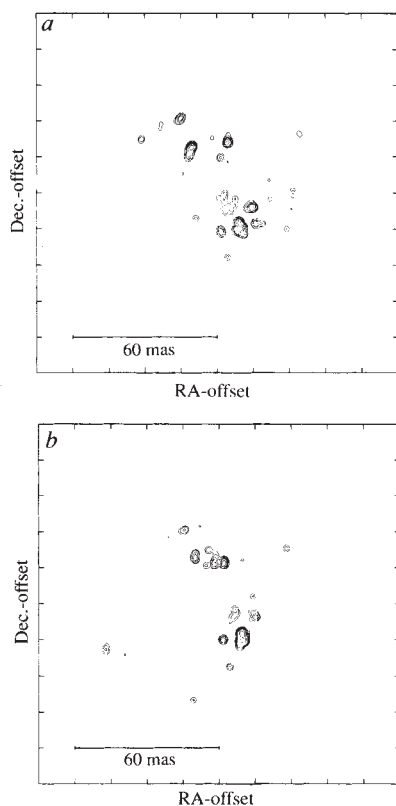


FIG. 2 Spatial distributions of SiO maser emissions integrated from $v_{\text{LSR}} = 15.05 \text{ km s}^{-1}$ to $v_{\text{LSR}} = 28.96 \text{ km s}^{-1}$ for VY CMa for the $J=1 \rightarrow 0$, $v=1$ (*a*) and the $J=1 \rightarrow 0$, $v=2$ (*b*) transitions. The contour levels are 100, 60, 35, 20, 10, 5, 3, 2, 1, 0.5 and 0.25 per cent of the peak. Gaussians of 2 mas half-power beam width which is narrower than the synthesized beamwidth ($\sim 3 \text{ mas}$ in α (right ascension, RA) and 12 mas in δ (declination, dec.) are used to show clearly the positions of the maser spots.

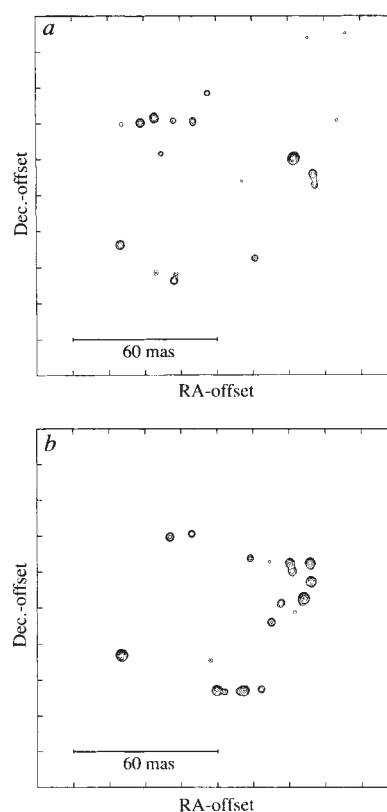


FIG. 3 Spatial distributions of SiO maser emissions integrated from $v_{\text{LSR}} = 33.79 \text{ km s}^{-1}$ to $v_{\text{LSR}} = 47.69 \text{ km s}^{-1}$ for W Hya for the $J=1 \rightarrow 0$, $v=1$ (*a*) and the $J=1 \rightarrow 0$, $v=2$ (*b*) transitions. The contour levels are 100, 60, 35, 20, 10, 5, 3, 2 and 1.5 per cent of the peak. Gaussians of 2 mas half-power beamwidth are also used. The synthesized beam is almost the same as that of VY CMa observations.

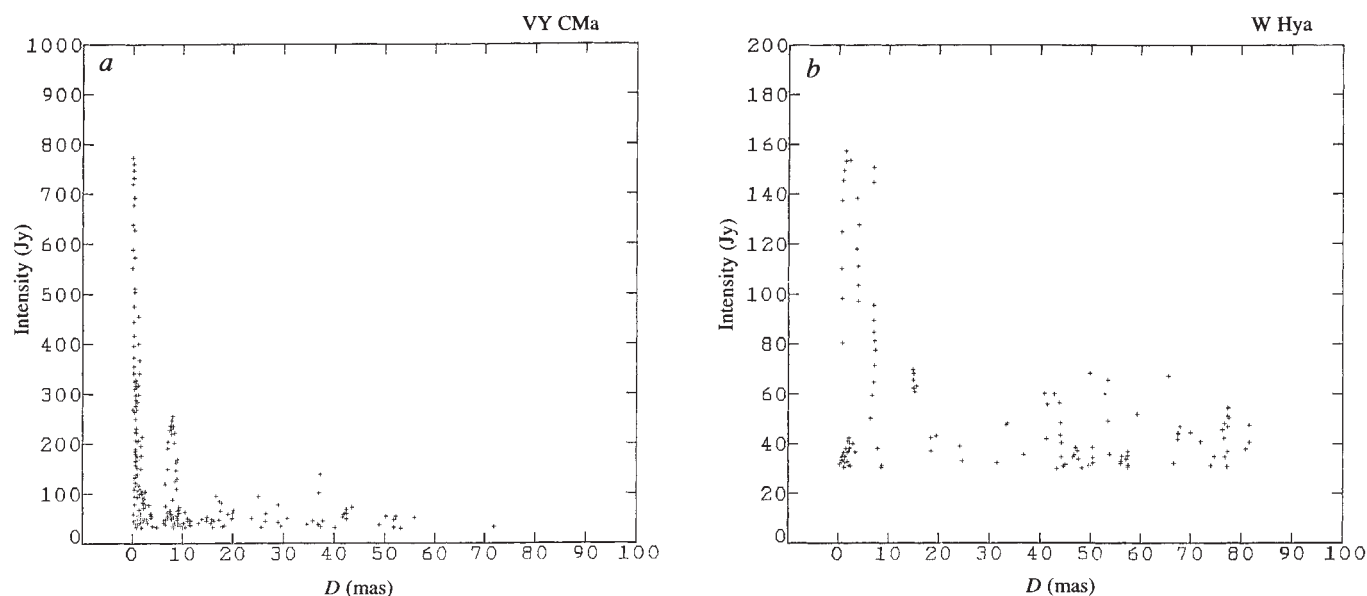


FIG. 4 The relation between the intensity of $v=1$ spots (y-axis) and the angular distance (D) to the nearest $v=2$ spot with the same velocity (x-axis), for VY CMa (a) and W Hya (b). The statistics were taken from the overlaid maps of the $v=1$ and $v=2$ emissions, assuming that the

reference features are in the same positions. These references were at $v_{\text{LSR}} = 22.17 \text{ km s}^{-1}$ in VY CMa and at $v_{\text{LSR}} = 33.18 \text{ km s}^{-1}$ in W Hya, and these channels are not included in the statistics.

SiO maser regions of VY CMa. As the spectral range of the emission from VY CMa was wider than our video-channel bandwidth, our maps missed some emission. Presumably the total size of the entire SiO-emitting region should be slightly larger than this stellar size. The SiO masers of W Hya exhibit a ring-like structure with a diameter slightly larger than the stellar size measured⁹ at a wavelength of 1.3 cm (90 mas, or 11 AU assuming the distance of 125 pc). The positions of the central stars are not certain in these VLBI maps. The size of SiO emitting region being comparable to the star, however, should mean that the stars are located at the centres of the SiO-emitting regions, implying that the SiO masers occur within one or two stellar radii of the stars. Recent very-long-baseline array (VLBA) observations (P. Diamond and L. Greenhill, manuscripts in preparation) show that ring structure in the SiO emission, like that observed here for W Hya, is the common shape of SiO in evolved stars. The different shape of the SiO-emitting region VY CMa may lend support to the suggestion that it is a pre-main-sequence young object¹⁰.

To analyse the positional coincidence between the $v=1$ and $v=2$ spots quantitatively, we have made intensity-distance diagrams for VY CMa and W Hya (Fig. 4a and b, respectively). These show the relation of the distance from each $v=1$ CLEAN spot to the nearest $v=2$ CLEAN spot with the same velocity. In VY CMa, 73% of the total $v=1$ emission ($1914.4 \text{ Jy km s}^{-1}$) comes from within the 2 mas regions around the $v=2$ spots with the same velocity. In W Hya, 25% of the $v=1$ total emission ($=367.6 \text{ Jy km s}^{-1}$) occurs within 2 mas of

the $v=2$ spots with the same velocity. Within the mapping accuracy, the $v=1$ masers have virtually the same positions with the $v=2$ masers.

The positional coincidence of maser spots in the $J=1 \rightarrow 0$, $v=1$ and $v=2$ SiO masers provides severe restrictions on the pumping mechanisms and the physical conditions of SiO masers. Radiative and collisional pumping schemes have both been proposed for SiO masers^{11–13}. It is known that in a smooth stellar wind neither radiative nor collisional pumping can yield a maser as strong as observed¹⁴. In very dense clumps (molecular hydrogen number density $n_{\text{H}_2} = 10^9\text{--}10^{10} \text{ cm}^{-3}$) close to the central star, both pumping schemes can produce SiO masers of strengths comparable to those observed¹⁵. In this situation, however, the standard radiative pumping has difficulty producing strong $J=1 \rightarrow 0$ masers in both $v=1$ and 2 at the same position in space, because the higher energy required to excite the molecules to the $v=2$ level ($T=3,570 \text{ K}$), compared to that needed for excitation of the $v=1$ level ($T=1,785 \text{ K}$). Obviously, our observations rule out this simple radiative pump mechanism. In the collisional pumping scheme, there is a significant common range of column density where both the $v=1$ and $v=2$ SiO lines can be produced¹⁵, and where the two lines can arise from the same physical conditions. The model also shows that the peak intensity of the $v=2$ maser occurs at column density ~ 30 times that of the $v=1$ maser¹⁵. Provided that this collisional pumping scenario and the present observations are right, the circumstellar gas of SiO maser regions must have extremely clumpy distributions. $\square$

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Identification of the guest star of AD 185 as a comet rather than a supernova

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VERY few nearby supernovae have been bright enough to see with the naked eye. The only such case this century was supernova 1987A. Matching historical records of such events with presently observable remnants allows accurate estimates to be made of the age and incidence rate of supernovae; ancient Chinese astronomical records are a particularly valuable resource for this purpose. The *Houhanshu*¹ of the Later Han dynasty records the appearance of a 'guest star' in AD 185. This is widely regarded as the oldest supernova recorded historically, and several candidate remnants have been suggested^{2,3}, in particular the object RCW86². Here we show that a reinterpretation of the relevant passage in the *Houhanshu* is inconsistent with the supernova interpretation, but suggests instead that the guest star was a comet. Our findings indicate that some of the keywords used by Chinese astronomers in historical records must be interpreted with caution.

On 7 December AD 185, astronomers at the imperial observatory of Lo-Yang in central China reported the appearance of a 'guest star' in the southern sky. This event was recorded originally in *Houhanshu*¹, the official history of the Later Han dynasty (AD 23–220). Here we translate the original record:

中平二年十月癸亥客星出南門中大如半筵五色喜怒稍小至後年六月消占曰為兵至六年司隸校尉袁紹誅滅中宮大將軍部曲將吳匡攻殺車騎將軍何苗死者數千人

"In the second year of the *Zhongping* reign period, the tenth month, on the day *Guihai* [7 December, AD 185], a 'guest star' emerged from the middle of the asterism *Nanmen* [Southern Gate]. It seemed to be as large as half a *yan* [bamboo mat]. It displayed the five colors, and *xi* [pleasure] and *nu* [anger]. It decreased gradually in size and in brightness. In the sixth month of the next year [5 July to 2 August 186] it disappeared. According to the standard prognostication this means military action. In the sixth year of the same reign period [AD 189] Metropolitan Commandant *Yuan Shao* wiped out the eunuchs; an officer *Wu Kuang* of the General-in-Chief attacked and killed *He Miao*, the Chariot and Horse General, and several thousand people were killed."

On several points, our translation differs from that given by other authors^{2–4}. In contrast to previous articles, the description of the astrological forecast has also been translated, because it will be relevant for the following discussion.

The asterism (group of stars) *Nanmen* was the southernmost constellation visible in AD 185 from Lo-Yang (34.7° N), the capital of the Later Han dynasty. The asterism, which is no longer visible from this latitude, is widely believed to consist of the two bright stars β and α Centauri^{2,3}, although a few authors have suggested ϵ and β Cen^{4,5} or ϵ and α Cen⁶. As we show below, it probably consists instead of ξ and α Cen. The coordinates of the west star of *Nanmen* are given in a description of this asterism which appeared in *Lingtai miyuan*⁷, which was originally written about AD 600, and rewritten and corrected between AD 1049 and AD 1054. It was 137 *du* from the North Pole and 11 *du* east of γ Corvi (a circle was defined to be 365 $\frac{1}{4}$ Chinese angular unit *du*). These coordinates are consistent with those of ξ Cen in the eleventh century. A similar description was also collected in *Kaiyuan zhanjing*⁸. It was 130 *du* from the

North Pole and 14 *du* east of γ Crv, which is consistent with the coordinates at about 70 BC, when the original measurement was done. In a late reference⁹ (eighteenth century), the coordinates and magnitudes of these two stars were found to be: *Nanmen* west; right ascension (RA) 197° 27', declination (dec.) –52° 20', magnitude 4. *Nanmen* east; RA 221° 42', dec. –59° 30', magnitude 1. This is consistent with ξ and α Cen.

In previous work, the words *chu* and *zhong* were translated respectively as "appear", and "middle" or "within", according to the usage of modern Mandarin Chinese. However in both *Lingtai miyuan*¹⁰ and *Kaiyuan zhanjing*¹¹ there are lists of the ancient definitions of some keywords used by Chinese astronomers. According to these definitions, *chu* was used to describe *wei dang qu er qu*,

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which means "an object should not have left an asterism but did leave". *Zhong* meant that an object went through an east-west asterism almost in the middle, and was not near the stars in this asterism within $\sim 0.7^\circ$. The two words can thus be translated literally "emerge from" and "through the middle". With these definitions we cannot translate the original record in terms of a guest star that stayed within the asterism *Nanmen*. Instead, it must have been an object changing its position on the sky.

From the last argument, it seems clear that the guest star was a comet, and not a supernova. We can, however, still study the remaining part of the text to get more information. In the next two sentences the size and brightness of the object were mentioned. First, it seemed to be as large as half a *yan*, where the word *yan* (as in the translation used in other references^{2–4}) means a bamboo mat. On the other hand, *yan* could also be an ancient unit of length which was defined as 9 *chi* (old Chinese length scale)¹². Because *du* and *chi* are used interchangeably in ancient Chinese astronomical texts for the angular unit, the size of this guest star could be ~ 4 or 5° . The colour and brightness of this guest star was described with *wuse xi nu*. *Wuse* (five colours) might happen because of the low elevation of *Nanmen* and its guest star. *Xi nu*, because of its original meaning "pleasure and anger", was translated to be "scintillated"² or "fluctuating"⁴. *Xi* and *nu*, along with *mang* and *jiao*, were actually used by the Chinese astronomers to describe the brightness of stars^{10,11}. Analysing some descriptions of the planets that contain these four words allows an estimate of the magnitude of the new object: this guest star was probably not brighter than the five planets visible with the naked eye. Considering the low elevation it could have been about magnitude 3 to 4. This guest star decreased gradually in size and in brightness until it disappeared, as recorded in the next sentence.

When did this guest star disappear? For this event no definite date is given. Instead, the term *hounian liuyue* is used. *Liuyue*, as translated before, denotes the sixth month of the Chinese calendar. *Hounian*, after the usage of modern Mandarin Chinese, means the year after next. According to this translation the guest star disappeared between 24 June and 23 July 187. Clark and Stephenson² excluded the possibility that this guest star could have been a comet from this point, because a comet could not be observed that long (~ 19 months). This contrasts with our investigation of the usage in *Houhanshu*, which suggests that *hounian* was used for the next year, whereas the year after next would be *houernian*, which means "the next second year". Therefore *hounian liuyue* was probably used to mean the sixth month of the Chinese calendar (5 July to 2 August) in AD 186. The asterism *Nanmen* could not be seen, however, from Lo-Yang until after the sixth month. If the guest star were a supernova, it would have stayed at the same position and could therefore be observed only after the sixth month, but if it were a comet, it could have been observed both before and after this time, because it would not have stayed in *Nanmen*.

The second half of the historical text is an astrological forecast with its associated events. In ancient China, the main task of astrology was to forecast the future from astronomical events. Predictions recorded in the official history had to be consistent with the omens. In official astrology⁷ the forecast based on a guest star staying in *Nanmen* was totally different from that of a guest star leaving *Nanmen*. The first interpretation implied war waged by foreign countries whereas the second predicted war inside China. Here the text refers to fighting between generals in China, and is thus consistent only with a guest star that moved out from *Nanmen*.

Our discussion gives a clear version of events. On 7 December AD 185, in the morning before sunrise, a comet of magnitude 3–4 appeared in the southern horizon between the stars ξ and α Cen. Its tail pointed northwest and was $\sim 4\text{--}5^\circ$ long. In the following days it moved north out of this region and decreased in size and in brightness. By July of the succeeding year, it was no longer visible.

One further point must still be clarified. Why was the event recorded with the words *kexing*, guest star, instead of *huixing*, which specified cometary events? In the beginning we assumed this guest star was a comet without a visible tail, but this is contradicted by the size of the guest star. After our detailed study of the whole section of astronomy in *Houhanshu*, we found other descriptions of comets with the name *kexing*, along with descriptions of the lengths of their tails¹³. Furthermore, one event was recorded initially as a *kexing* and after several days as a *huixing*¹⁴. This word *huixing* used as the translation of “comet” in astronomical writing, is actually only a usage of modern Mandarin Chinese. Astrologers had special terms for comets with visible tails, but did not always feel the need to use them. Guest star was the common term for transient events such as comets and supernovae, where the meaning of the omen stressed its transience. It is not sufficient to identify a guest star by the word used for it; the context is also highly important.

Because only very few Galactic supernovae have been observed in the last two millennia, the identification of an historical supernova with an associated remnant is important for our understanding of supernova. Each individual case can be used to study the evolution of a supernova remnant after its formation; the supernova rate can be calculated only with these observed data. As mentioned above, RCW86 (alias MSH14–63, G315.4–2.3, PKS1439–62, Cen XR-1 and 1H1438–623) is widely taken to be the remnant of SN185 (ref. 2). Thorsett³, however, proposed that the counterpart of this supernova is PSR1509–58 in the supernova remnant RCW89 (alias MSH15–52 and G320.4–1.2), and it would be then only the second pulsar, after PSR0531+21 in the Crab nebula as the counterpart of SN1054^{15–17}, to have a known age. There are, however, further problems associated with this identification: Schaefer¹⁸ argued that such an identification would require too high a luminosity. A low value for the Hubble constant might also be required from the visibility constraints. Strom¹⁹ discussed the physical parameters (for example, distance, luminosity and visual magnitude) of these two remnants, and concluded that RCW86 must remain the preferred candidate for SN185. Nevertheless this identification with RCW86 remains disputed. For instance, it does not resemble a young supernova remnant of age 1,800 yr; 10^4 yr is more likely³. Furthermore, there is an additional problem with the supernova rate: from Poisson statistics and a Scalo initial mass function²⁰, van den Bergh and Tammann²¹ determined the probability that one or more core-collapse supernovae have occurred within 1 kpc of the sun (where RCW86 is located²²). The probability is only 0.06 for $M_{\min} = 8M_\odot$, or 0.14 for $M_{\min} = 5M_\odot$ (where $M_{\min}$ is the minimal mass to form a core-collapse supernova), during the past 2,000 yr. These problems disappear, however, if there were no supernova in AD 185. On the other hand, Strom²³ discussed the local supernova rate with all the historical records and the observed young supernova remnants. Although one event less

might not change substantially the observed supernova rate, our discussion shows that a more careful investigation²⁴ of other historical records is necessary for a reliable determination of the local supernova rate. □

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Velocity dependence of collisional alignment of oxygen molecules in gaseous expansions

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THE orientational dependence of molecular interactions has long been recognized as central to an understanding of reaction mechanisms and of collisions in the gas phase and at surfaces. Studies of orientation effects have recently become possible owing to the development of techniques for aligning molecules. ‘Brute-force’ methods using electric or magnetic fields can induce alignment of molecules with dipole moments^{1,2}, and polarized-absorption approaches³ can be used in cases where there are suitable molecular transitions; but one of the simplest and most general methods involves the supersonic expansion of molecular beams seeded with molecules that induce rotational alignment—selection of specific rotational states—by collisions^{4–12}. Here we use such an approach to induce strong rotational alignment of oxygen molecules in a beam seeded with various other gases at close to atmospheric pressure. Most significantly, we find that the degree of alignment depends on the velocity of the molecules in the supersonic expansion—fast molecules are much more highly aligned than slower ones, and the velocity of maximum alignment can be altered by changing the gas mixture. In this way, we can prepare rotationally aligned molecules with well defined velocities, opening up new possibilities for experiments in molecular dynamics.

When gaseous mixtures expand into vacuum through holes having diameters greater than kinetic mean free paths, the heavier components are accelerated to supersonic speeds higher than when pure: this 'seeding' phenomenon leads to cooling of molecular degrees of freedom and also—as shown for Na_2 , Li_2 , I_2 and CO_2 (refs 1, 2, 4–9 and 10, respectively) by laser probing of selected states within their dense rotational manifold—to spatial alignment. This collisional alignment of molecules, qualitatively anticipated long ago¹³ in connection with a transport phenomenon (the Senftleben–Beenakker¹⁴ effect), is usually interpreted using classical models. The phenomenon is illustrated in Fig. 1, which also introduces some relevant notation, such as the rotational angular momentum $\mathbf{K}$ (only odd K -values allowed for O_2 which has no nuclear spin) and the helicity M , that is, its projection along the velocity $\mathbf{v}$ (positive and negative helicities only differ by the sense of rotation, and therefore only absolute values for M are relevant under the axial symmetry of the expansion). The cooling and alignment of molecular rotations are the results of several collisions with the seed gas, and depend on the cross-sectional area that a particular rotational state presents perpendicular to the flow direction. Figure 1 depicts cases of collisions with low, intermediate and large impact parameters: in a quantum-mechanical picture, which is more appropriate to the description of present results (see below), correspondence must be made to low, intermediate and large orbital angular momenta and collisions are classified as falling into cases γ , δ and ε respectively (this classification is reminiscent of the Hund's cases of spectroscopy^{15–17}). For room temperature O_2 , rotational states are distributed over a range of K values (the most probable being $K=9$) and random helicities M : they will be collisionally deactivated to lower K states only when the helicity is sufficiently low. A substantial variation in the helicity quantum number is a very slow process which requires many collisions: in fact M is 'good' in case γ , and its decrease requires case δ (that is, intermediate angular momentum) collisions.

As we will see, the final rotational state in our experiment can be as low as the ground state $K=1$. Oxygen molecules also have spin $S=1$, which adds to K to give three possible values for the total angular momentum $J=0, 1, 2$ in the ground state. The resulting paramagnetism is here exploited by a magnetic deflection technique (Fig. 2) which is used only to analyse the product state, not as an essential part of the orientational process: care is taken that the passage of molecules from zero field outside the magnet to high field inside occurs suddenly, to avoid non-adiabatic transitions and effective depolarization, apart from the natural one due to K – S coupling. The same apparatus had been used for the diagnostics and scattering of fine-structure states in oxygen^{18,19}, fluorine^{20,21} and chlorine^{22–24} atomic beams.

With this apparatus, we have made several studies of thermal and superthermal effusive beams of O_2 , and these will be

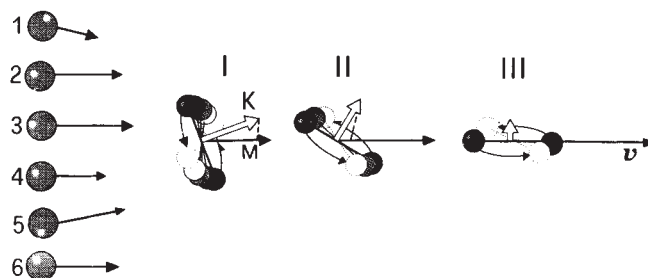
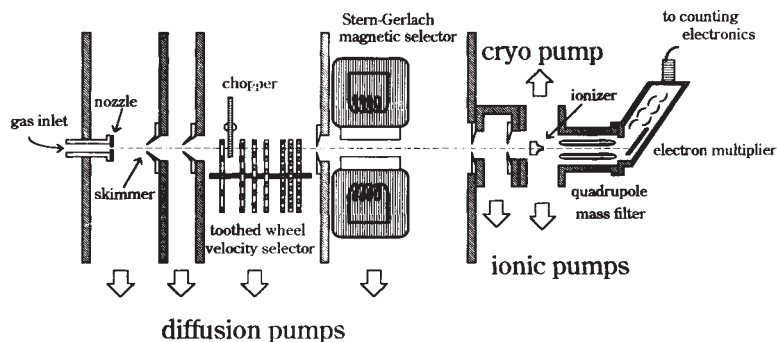


FIG. 1 A classical view of acceleration, rotational cooling and alignment in expansions of diatomic molecules seeded in a lighter (monatomic) gas. A diatomic molecule (as in I) rotating in a plane randomly oriented with respect to the flight direction $\mathbf{v}$ expands into vacuum at sufficiently high pressure that it experiences several collisions with faster atoms of the lighter seeding gas. $\mathbf{K}$ is the rotational angular momentum, and M its helicity (that is, its projection along $\mathbf{v}$). Collisions with impact parameters b of the order of a molecular dimension or smaller (for examples with atoms 3 and 4) lead to acceleration (exchange of linear momentum and increase in $\mathbf{v}$), whereas those with intermediate b (atoms 2 and 5) may also bend the rotational plane, with a net decrease in the helicities M (a bottleneck requiring many collisions, see text). Collisions with large b (atoms 1 and 6) will only elastically deflect the molecules, focusing them in the forward direction. After several collisions, most molecules (as in II) will have low helicity; that is, they will travel edge-on, offering a target for collisional removal of rotational angular momentum. Those molecules that have suffered a sufficiently high number of collisions will be as in III, that is fast, very slowly rotating and with such a low helicity as to be mainly aligned along $\mathbf{v}$ (as can be seen by imagining the (now small) vector $\mathbf{K}$ distributed uniformly on a plane orthogonal to $\mathbf{v}$).

reported elsewhere. The distributions of rotational levels in general involve several K states, but in seeded beams, and at total stagnation pressures of ~ 1 atm, efficient cooling can occur. Specifically, from measured velocity distributions (see Fig. 4) the relevant translational temperatures are found to be so low (~ 3 K) that in the analysis only $K=1$ need be considered (the next level, $K=3$, lies ~ 20 K higher). Such a correlation between final rotational distributions and the transverse translational temperature had been established by extensive previous studies of both pure and seeded O_2 beams^{25–28}.

Our measurables are beam intensities I and I_0 with and without applied magnetic field, and the working equation is $I/I_0 = \sum_{Jm_J} T_{Jm_J} w_{Jm_J}$, where w_{Jm_J} are relative populations of specific sublevels, labelled by the total angular momentum J and its projection m_J along the magnetic field direction. The

FIG. 2 A diagram of the apparatus (not to scale; overall length ~ 2 m) for the production of molecular beams, their velocity and magnetic analysis, and detection. Mixtures of oxygen molecules diluted in various gases of lower mass at typical stagnation pressures of the order of 1 atm enter through a nozzle of diameter ~ 0.1 mm in a first vacuum chamber. In the region between nozzle and skimmer (the latter having a diameter ~ 1.2 mm), the supersonic oxygen molecules are translationally accelerated but rotationally cooled by the seeding phenomenon. The nozzle–skimmer distance can be varied, and is of the order of 10 mm. Then, in a collision-free flight through an intermediate collimation chamber and a defining hole, the beam proceeds to the velocity selector vacuum chamber, where seven rapidly spinning slotted disks transmit a fraction of the beam (velocity selected to within $\pm 20 \text{ m s}^{-1}$) into a vacuum chamber where magnetic deflection takes place. The detector housing, which is preceded by an intermediate chamber, contains an



electron bombardment ionizer, a quadrupole mass spectrometer and an electron multiplier.

empirical transmission factors T_{Jm_J} (see Fig. 3 and also ref. 22) account for the characteristics of the deflecting magnet, for the geometrical features of the acceptance slits to the detector and for the effective magnetic moments μ : the latter are also shown in Fig. 3 as have been calculated according to the relevant literature²⁹. The procedure is to measure I/I_0 for selected beam velocities as a function of the applied magnetic field in order to obtain the population weights w_{Jm_J} . The relative populations w_m for projections m of the rotational angular momentum $K=1$ along the magnetic field direction are then calculated from the formula $w_m = \sum_{Jm_J} \langle Km, Sm_s | Jm_J \rangle^2 w_{Jm_J}$, where $\langle \dots | \dots \rangle$ are vector coupling coefficients. Rather than m , physically more interesting quantities are the rotational helicities M (see Fig. 1); as the magnetic field and velocity directions are orthogonal, a consideration of appropriate rotation matrices for a $K=1$ rotational angular momentum vector allows the calculation of the relative weights for $M=0$ and 1 (either +1 or -1). These weights are indicated as $w_\perp$ and $w_\parallel$ respectively, because in a classical picture $M=0$ or 1 correspond to oxygen molecules travelling edge-on or broadside, respectively, with their rotational angular momentum perpendicular or parallel to the direction of travel.

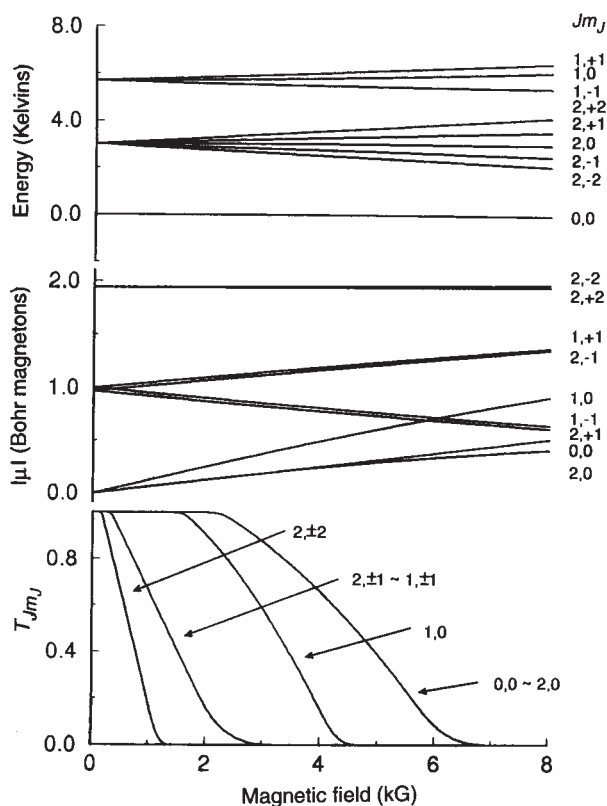


FIG. 3 Magnetic-field dependence of energy levels of oxygen molecules (in kelvin units, 1 kelvin = 8.314 J mol^{-1}) (top), their effective magnetic moments (middle) and their transmission factors $T_{Jm_J}(v)$ (bottom) in our apparatus (the $J=0$ level at zero magnetic field is taken as the origin for the energy scale). The resulting Jm_J states have the effective magnetic moments $|\mu| = |\partial E / \partial B|$ shown in the middle panel—the sign of μ is irrelevant for total deflection measurements. From $|\mu|$ and from the geometrical features of our apparatus, we calculate how molecules with given Jm_J are deflected and thus their transmission factors $T_{Jm_J}(v)$, shown in the lower panel for a typical velocity of the beam $v = 0.78 \text{ km s}^{-1}$. The $T_{Jm_J}(v)$ s are in four groups. This grouping assists the analysis needed to obtain proper combinations of relative populations w_{Jm_J} of substates from the magnetic-field dependence of the measured beam transmittance. So, for example, the observed deflection at this velocity is mainly due to the $2, \pm 2$ states for $B \leq 1 \text{ kG}$, whereas states with $m_J = 0$ are only deflected for $B \geq 3 \text{ kG}$.

A sample of the obtained results is shown in Fig. 4, which shows the dramatic final speed dependence of the 'alignment polarization fraction', conveniently defined as $P = (w_\perp - w_\parallel) / (w_\perp + w_\parallel)$. At low velocities, no substantial alignment is apparent ($w_\perp \approx w_\parallel$, or $P \approx 0$); as the velocity increases, the observed positive values of P are an indication that $w_\perp > w_\parallel$. Therefore molecules with the rotational plane aligned along the direction of flight prevail for the faster portion of the beam, while the slower ones are more randomly distributed. Two of the examples shown illustrate that the effect increases with the 'velocity slip' factor^{1,5-8,10}, that is lighter seeding gases induce higher overall polarization; the third example proves that a mixture of seeding gases leads to intermediate effects. Going from left to right for the three cases in Fig. 4, $w_\perp/w_\parallel$ is found to rise from 1 in the tail of the beams to 4.3 ± 1.5 , 6.3 ± 2.0 and 10.4 ± 3.5 , respectively, for molecules travelling ahead in the beam. The ratios $w_\perp/w_\parallel$ at peak speeds (1.8 ± 0.5 , 1.9 ± 0.5 and 2.3 ± 0.5) are useful for comparison with previous findings: they can be shown to correspond closely to what one obtains averaging over the velocity distributions, and these values are similar to those found in refs 7 and 10.

Mixtures of diverse compositions were typically studied at $\sim 1 \text{ atm}$ (at higher pressure, problems arise because of pumping speed requirements and possible cluster formation). A systematic investigation of the pressure dependence shows that down to $\sim 100 \text{ torr}$ the effects are qualitatively the same, although progressively attenuated; at lower pressures the cooling is less extreme, and $K=3$ and higher rotational levels can also be populated.

The role of other seeding gases was studied systematically. Molecular hydrogen gave effects as large as helium. Although less spectacular, the effects also occur in air (a 20% mixture of O_2 in N_2 at $\sim 1 \text{ atm}$ pressure). To illustrate the general nature of the phenomenon we record that, after expansion, oxygen is accelerated from thermal to supersonic with Mach number ~ 15 (peak velocities from 0.48 to 0.77 km s^{-1}) and polarization P rises from zero ($w_\perp/w_\parallel = 1$) to 0.26 ± 0.10 ($w_\perp/w_\parallel \approx 1.7 \pm 0.5$) at

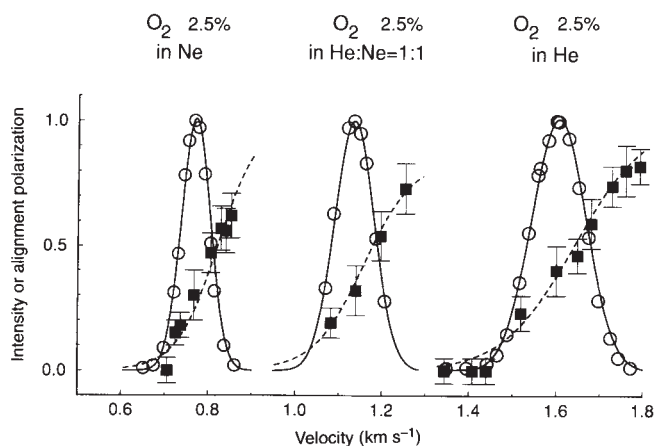


FIG. 4 Degree of alignment (see text) and velocity distributions for seeded beams of oxygen molecules in (from left to right) Ne, a He-Ne mixture, and He at a total pressure of 800 torr. Open dots indicate relative beam intensities of O_2 molecules and black squares indicate their alignment polarization fractions P as a function of velocity. The velocity distributions have shapes typical of supersonic beams and peak at values much higher than 0.5 km s^{-1} , the most probable velocity for an effusive beam at room temperature. The continuous curves are the best-fit velocity distributions obtained by using Mach numbers of 22 (He), 18 (He-Ne mixture) and 17 (Ne), which correspond to translational temperature values between 2 and 3 K. The dotted curves are a visual aid to the velocity dependence of the alignment polarization fractions P .

the peak speed, and to 0.50 ± 0.10 ($w_{\perp}/w_{\parallel} \approx 3.0 \pm 1.0$) for molecules travelling in front of the beam at 0.85 km s^{-1} .

These observations, which stimulate the quantum-mechanical extension of previous classical models, prove that we have a powerful and general tool to produce stereodirected states¹⁷, to study steric effects in collisions of molecules in gases and on surfaces. By varying the composition and partial pressure of the mixtures, beams can be generated in which molecules such as oxygen travel with an alignment that can be varied in a controlled way, from random to pure edge-on mode. This allows the realization of collisional configurations for which an interesting phenomenology and nomenclature is already being introduced: the 'edge-on' or 'broadside' molecules will give 'cart-wheel' or 'helicopter' states when impinging on surfaces^{30,31}, and 'pinwheel' or 'airplane' states when colliding with other molecules¹⁷. □

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Disordered waves in a homogeneous, motionless excitable medium

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EXCITABLE media are physical, chemical or biological systems in which energy dissipation in disturbed regions is compensated by energy supply^{1–4}, so that the media can support waves without attenuation. Well-known examples are nervous tissue, heart muscle, retinae, aggregating amoebae and the autocatalytic Belousov–Zhabotinsky (BZ) reaction. Most familiar in such systems are periodic target and spiral waves, but a number of simulations^{5–11} have suggested that disordered waves can also occur in a homogeneous and motionless excitable medium. In all experimental observations reported so far, however, hydrodynamic flow^{12,13} or inhomogeneities^{14,15} were necessary to induce disorder. Here we present experimental evidence for disordered chemical waves in a convection-free and homogeneous medium, a gel containing a light-sensitive BZ reaction mixture. We find instabilities transverse to the wavefront ('ripples'), wave breakup leading to aperiodic spiral formation, labyrinthine structures, and erratic motion of non-spiralling wave fragments. The formal analogy of

the BZ reagent with other excitable media suggests that this disordered behaviour may be generic.

We investigated the BZ reaction catalysed by the ruthenium bipyridyl complex $[\text{Ru}(\text{bpy})_3]^{2+}$, which is sensitive to visible light, thus allowing external control of the system^{16–19}. We observed spatial disorder when using extremely low Ru^{2+} concentrations, namely 0.71 mM, as compared with 2.5–4 mM in other work. The Ru^{2+} complex was immobilized in a silica-gel matrix (using 15% sodium silicate solution; preparation as in ref. 20) in a Petri dish (diameter, 6.2 cm; thickness, 0.9 mm). The solution (initial concentrations; 0.18 M NaBr, 0.33 M malonic acid, 0.39 M NaBrO_3 and 0.63–0.87 M H_2SO_4) was poured onto the gel. As the volumes of this solution and the gel were equal, the concentrations decreased to one-half of their initial value. The temperature was kept at $25 \pm 1^\circ \text{C}$. White light (250 W halogen lamp with digital intensity regulation) passed first an interference filter (450.6 nm; transmission, 56%), then a diffusion screen, then the Petri dish, and finally video equipment for image recording.

The initial conditions were periodic waves (a spiral pair extending over the whole Petri dish), which were allowed to develop in the dark for 2 hours. Destabilization of these periodic waves was then observed after illumination. The experimental conditions were found after careful optimization in control parameter space. An initial concentration of H_2SO_4 below 0.6 M gave too rapid wave breakdown, whereas above 0.87 M the required light intensities were higher than those our lamp could yield. The concentration of H_2SO_4 could be used to control the

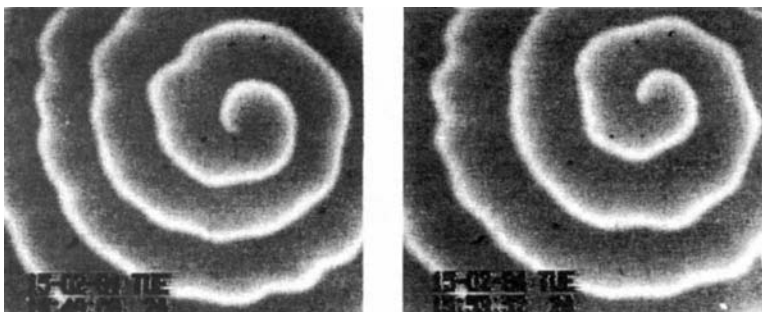


FIG. 1 Spiral with 'ripples' resulting from a wavefront instability. Times, 0 (left) and 4.73 min (right); mean spiral period, 1 min; light intensity, 280 mW m^{-2} ; frame widths, 12 mm.

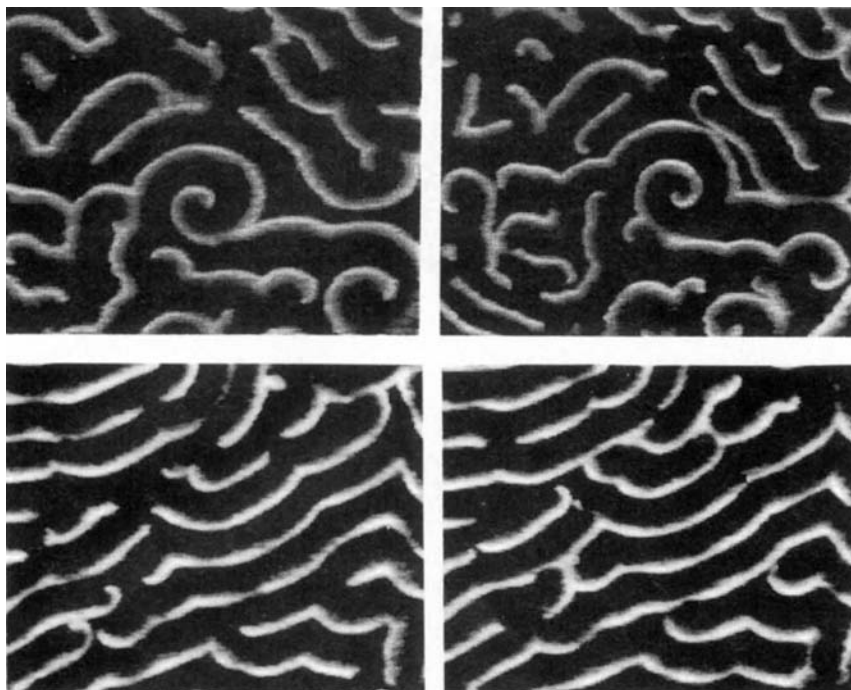


FIG. 2 Aperiodic wave breakup at 430 mW m^{-2} light intensity. Top row, multiple spirals at 0 (left) and 1.4 min (right); frame widths, 25 mm. Bottom row, labyrinthine patterns at 0 (left) and 0.37 min (right); frame widths, 22 mm.

average wavelength of the travelling fronts (2.5 mm for 0.63 M, down to 1.5 mm for 0.87 M H_2SO_4), leaving other wave properties essentially unchanged. As to $[\text{Ru}^{2+}]$, we found fast destruction of waves for values below 0.55 mM, approximately constant results between 0.7 and 0.9 mM, and suppression of wave breakup for values larger than 1.2 mM. Gel thicknesses below 0.7 mm could not be set without large thickness inhomogeneities; values increasing between 0.9 and 1.3 mm required increasing light intensities, but show no qualitative change in the dynamics. As to $[\text{NaBrO}_3]$, we found fast destruction of waves below 0.33 M and no wave breakup above 0.43 M. Instabilities

did not appear for durations of initial spiral development (dark period) below 30 min or above 2.5 hours.

After increasing the light intensity (at a rate of $30\text{--}70 \text{ mW m}^{-2} \text{ min}^{-1}$ to values below 300 mW m^{-2} the spirals were distorted by wavefront instabilities leading to aperiodic 'ripples' (Fig. 1). Similar instabilities have been described theoretically, assuming kinetic interaction of the wave with a reactant in front of it²¹ or assuming lateral inhibition²². But to our knowledge, the physical chemistry of the Ru^{2+} catalysed BZ reaction in a gel is not yet sufficiently well known to explain these instabilities. The spiral distortions became visible 3–10 min after starting to

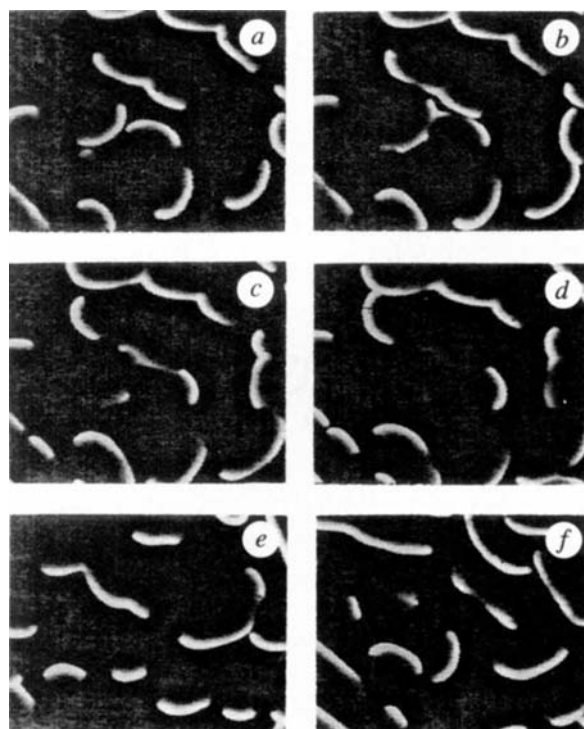


FIG. 3 'Frazzle-gas' at 580 mW light intensity. Times of images (s): a, 0; b, 0.25; c, 0.41; d, 0.55; e, 12.78; f, 21.50 min. a–d are close enough in time to see the temporal development in detail (wave fusions and wave breaks), while e and f show how well the overall spatiotemporal disorder is sustained in time. Frame widths, 17 mm.

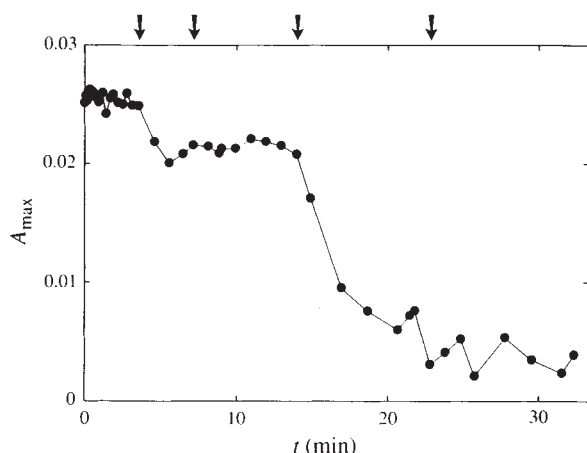


FIG. 4 Time dependence of the maximum correlation function $A_{\max}$, as light is increased during the displayed time range at a slow, constant rate ($30 \text{ mW m}^{-2} \text{ min}^{-1}$). No destabilization of the initial spirals is visible earlier than $t = 4 \text{ min}$ (first arrow), as indicated by a high, constant $A_{\max}$ (~ 0.025). Afterwards, $A_{\max}$ drops, as ripples develop. Ripples reach a constant mean amplitude for $7 < t < 14 \text{ min}$ (between the second and the third arrow), as characterized by a locking of $A_{\max}$ to a new, constant value (~ 0.021). Then, $A_{\max}$ drops again as an increasing number of breakups occur. Finally, a 'frazzle-gas' develops; this dynamic state is characterized by $A_{\max}$ fluctuating around a constant value (~ 0.003 ; after the fourth arrow). Frame, $12 \times 12 \text{ mm}$ (100×100 pixels).

increase illumination, and the resulting ripples lasted up to 2 hours. If, after ripple formation, light intensity was further increased to values above 400 mW m^{-2} , then wave breakups occurred at some concavity maxima (ripple valleys). In contrast, if the light intensity was increased from zero to values above 400 mW m^{-2} at a rate of $200 \text{ mW m}^{-2} \text{ min}^{-1}$, then breakups occurred without previous ripple formation, at disorderly distributed points on the wavefronts, as in simulations^{5,9}. Depending on the procedure, we could thus obtain the two instabilities independently: ripples without breakups, and breakups without ripples.

As causes of the observed ripples and breakups, we discard inhomogeneities of the gel or the illumination, because the dynamics did not change at any stage on displacement of the dish within the light beam; also, ripples or breakups appeared at different points when re-initiating experiments after erasure of the patterns by strong light, or after replacement of the reactive solution. The effect of the gel porous structure (typical lengths below $1 \mu\text{m}$; ref. 23) is excluded because our typical lengths are more than three orders of magnitude larger. As far as we know, there is no correlation between the patterns formed during the preliminary dark period and the points at which instabilities developed after illumination; in fact, we could detect no devia-

tions from periodicity at the end of the dark time (and for a few minutes after illumination), but ripples and breakups thereafter were scattered in a disordered fashion. Furthermore, we discard instabilities due to interaction of the solution with the gel (as described in ref. 23) because they were shown not to appear at the low concentrations (15%) of sodium silicate used here; however, any future model may have to consider how the reaction works when embedded in a gel.

After wave breakup, the following modes occur. (1) At large enough spacing between wavefronts, spirals develop, get aperiodically disrupted yielding new spirals, and so on (Fig. 2, top row; note the similarity with simulations^{5,8}). (2) At small enough spacing between wavefronts, spirals fuse with other fronts after only slight bending (aperiodic labyrinthine patterns; Fig. 2, lower row). (3) At large enough light intensities, the excitability of the medium is so low that no spirals are formed (as in the weakly excitable cerium-catalysed reaction²⁴), and one obtains aperiodic motion of uncurling wave pieces ('frazzle-gas'; Fig. 3). The modes (1)–(3) are reminiscent of observations in a medium containing cation-exchange beads loaded with catalyst¹⁵. In that work, however, disordered waves are clearly caused by inhomogeneities (bead sizes, $0.1\text{--}1 \text{ mm}$), whereas in our experiments there are no indications of relevant deviations from homogeneity.

In order to quantify disorder, we calculated the spatial correlation function $A(j) = \langle \delta I(i) \delta I(i, j) \rangle$, as in ref. 15. The quantity δI is the difference between the intensity and its average over all pixels, $\delta I(i, j)$ is the average of δI in an annulus three pixels wide around pixel i at radius $j \pm 1$ and the average $\langle \rangle$ is taken over all pixels i . Figure 4 shows the temporal evolution of the first maximum ($A_{\max}$) of $A(j)$, numerically characterizing the formation of ripples and of a 'frazzle-gas'. After full development of these dynamic modes, $A_{\max}$ fluctuates around constant values, suggesting the occurrence of attractors.

Simulations of spiral breakups in excitable media have been related to cardiac fibrillation^{5,9} (uncorrelated muscle contractions leading to rapid death). These simulations could be comparable with our results by virtue of the analogy between reductionistic differential equations for the BZ reagent¹⁹ (excitation variable, HBrO_2 ; recovery variable, Ru^{2+} immobilized by the gel) and for the neurophysiology of the heart³ (excitation variable, local electrical potential; recovery variable, conductivity of membrane ionic channels involved in repolarization). However, more has to be known about fibrillation to build on such an analogy (see ref. 25). In any case, the present observations offer the novelty that inhomogeneities or flow are not necessary to produce aperiodic waves in an excitable medium; disorganization may thus be the result of purely reaction-diffusion processes. Thus one may expect such aperiodicities in other excitable media. Furthermore, this work suggests the use of the BZ reaction as a simple prototype system to test general methods for global analysis^{13,15,26}, as well as control²⁷, of spatiotemporal disorder. □

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Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea

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THE export of biogenic carbon from the upper ocean is responsible for maintaining the vertical gradient of dissolved inorganic carbon and thus indirectly for regulating the level of atmospheric CO₂ (ref. 1). Large, rapidly sinking particles are thought to dominate this export², and this sinking flux has been thought to balance new production³. Recent measurements of particle export^{4–6} and estimates of new production^{7–9} have questioned this picture, however. Here we report measurements of dissolved organic carbon (DOC) off Bermuda, which provide strong support for the idea^{10–15} that this component of oceanic carbon is also an important and dynamic part of the ocean carbon cycle. We find that DOC accumulates in the early spring owing to increased primary production, and is partially consumed in the summer and autumn. The DOC that escapes remineralization is exported from the surface ocean the following winter, and we estimate this export to be equal to or greater than the measured particle flux, allowing us to close the annual vertical carbon budget for this site to within a factor of two. Our observations should be applicable to other temperate, sub-polar and continental-shelf regions of the world ocean which exhibit convective mixing and vernal restratification.

Renewed interest in DOC was sparked by a series of reports of elevated concentrations determined by the high-temperature combustion (HTC) method^{14,16} suggesting the presence of a previously unmeasured and chemically refractory pool. Although

these elevated DOC values have since proven to be incorrect^{17,20}, the controversy stimulated efforts to refine the HTC method, improving the precision and accuracy of the analysis of DOC in marine systems^{20–22}. Our average analytical precision is now <2% (comparable to $\pm 1.1 \mu\text{M C}$). This improved precision enables us to detect small changes in DOC concentrations and distributions and assess the role of DOC in global carbon cycling.

The samples were collected in the vicinity of the US JGOFS Bermuda Atlantic time-series study (BATS) station and Hydrostation S in the western Sargasso Sea. This site has strong seasonal patterns of mixing and biological production, dominated by a spring phytoplankton bloom^{5,6,23}. As in any oceanic system, mesoscale eddies may bias a discrete sample time series²⁴. We attempted to reduce the influence of mesoscale features on our sampling programme by making repeated measurements over periods of weeks within each season (except in autumn 1991 and winter 1992). This should allow our average seasonal profile to represent an integration of the mesoscale variability within each season (sampling programmes are detailed in Figs 1 and 2).

Vertical profiles show seasonal variability of DOC and temperature in the top 250 m (Fig. 1). The spring 1992 data were gathered during late bloom conditions. A broad subsurface DOC maximum developed between 40–60 m when the water column had completely restratified (Fig. 1a, b). By summer 1992 and 1993, a surface mixed layer of ~20 m developed, within which DOC concentrations were distributed homogeneously. Below this mixed layer a subsurface maximum persisted between 40–50 m (Fig. 1a). In the autumn of 1991 and 1992, the surface mixed layer deepened to ~50 and 60 m respectively (Fig. 1b) and DOC was distributed homogeneously within the mixed layer. DOC decreased sharply from just below the mixed layer to 250 m. The sampling programmes in winter 1992 and 1993 were concurrent with a deep mixing event and mixed layer depths ranged from 160–260 m (Fig. 2b) within which DOC was homogeneously distributed.

The covariance of DOC profiles with the vertical structure of temperature reflects the influence of biological and physical processes, which govern the distribution of DOC. The subsurface maxima observed in the spring and summer DOC profiles indicate an accumulation of DOC due to an uncoupling of production and consumption. In the spring and summer the structure of the DOC profile differed greatly from that of temperature

TABLE 1 A carbon budget for the upper ocean near Bermuda: a comparison of new and export production

Method	Measured parameter (mol m ⁻² yr ⁻¹)	Carbon equivalent (mol C m ⁻² yr ⁻¹)	Ref.
New production			
Oxygen geochemistry	~5 (O ₂)	2.9–4.16*	7
Nitrate flux via ³ He correlation	0.6 (NO ₃)	3.97†	8
Argon, helium, oxygen tracers	5.1 (4.3–5.6) (O ₂)	3.0–4.25*	9
Tritium box model		2.4–3.5	29
Nitrate flux estimated from 18 °C water ventilation	0.38 (NO ₃)	2.5†	27
Export			
Oxygen utilization rate‡	4.1–5.9 (O ₂)	3.3–4.72	7
Sinking PON (100 m sediment traps)	0.2 (PON)	1.32	4
Sinking POC§ (150 m sediment traps)	0.77–0.78 (POC)	0.77–0.78	5
Sinking POC§ (150 m sediment traps)	0.71 (POC)	0.71	6
Advected DOC	0.99–1.21 (DOC)	0.99–1.21	This study

Abbreviations used: PON, particulate organic nitrogen; POC, particulate organic carbon.

* Photosynthetic quotients ranging from 1.2–1.7 were used to calculate annual C production from O.

† C/N ratio of 6.63 was used to estimate annual C production from nitrate flux.

‡ O utilization rates integrated from 100–400 m were used to estimate the total C utilized below the euphotic zone. This value represents a total export of organic material out of the euphotic zone. A respiratory quotient of 0.8 was used to calculate the C utilization from O consumption.

§ Sediment traps were deployed at 150 m. Based on the ocean composite relationship of Martin and colleagues³⁰, one would expect the flux at 150 m to be comparable to 67% of 100 m flux yields.

in the surface 100 m suggesting that biogeochemical rather than physical processes regulated DOC distribution during these seasons. But there was a slight salinity maximum near 50 m in the summer and we cannot rule out the possibility that horizontal advection influenced the profiles. In the autumn and winter, the deepening of the surface mixed layers eradicated the DOC subsurface maximum. DOC appeared to behave conservatively during these seasons with physical mixing processes governing its distribution in the top 250 m.

The relative increases in DOC stocks ($\int$ DOC) within the surface 250 m during March 1991 and 1992 were coincident with phytoplankton blooms (Fig. 2a), indicating that DOC production was greater than consumption during these periods. Despite this production and accumulation, $\int$ DOC in the surface 100 m decreased compared to autumn concentrations (Fig. 2b). As a result of deep mixing, low DOC water was entrained into the

upper 100 m lowering the $\int$ DOC in this layer (Fig. 2b) and higher surface DOC was mixed below the euphotic zone elevating $\int$ DOC in the 100–250 m layer (Fig. 2c). Upon restratification of the water column in April 1992, there was further accumulation of 'freshly produced' DOC in the surface 100 m which increased $\int$ DOC (Fig. 2b) and produced a subsurface maximum (Fig. 1a). We observed a significant decrease ($P < 0.05$) in $\int$ DOC in the upper 100 m from April 1992 to March 1993 (Fig. 2b). From April to November 1992, the surface mixed layer remained well above 100 m eliminating the possibility of DOC removal by deep mixing; we attribute the observed decrease to biological consumption. Approximately $0.5 \text{ mol DOC m}^{-2}$ were consumed at a rate of $\sim 2.3 \text{ mmol C m}^{-2} \text{ d}^{-1}$ in the top 100 m from April to November 1992. The decrease in $\int$ DOC observed from October 1991 to March 1992 and November 1992 to March 1993 (Fig. 2b) was due to dilution of high DOC water by the

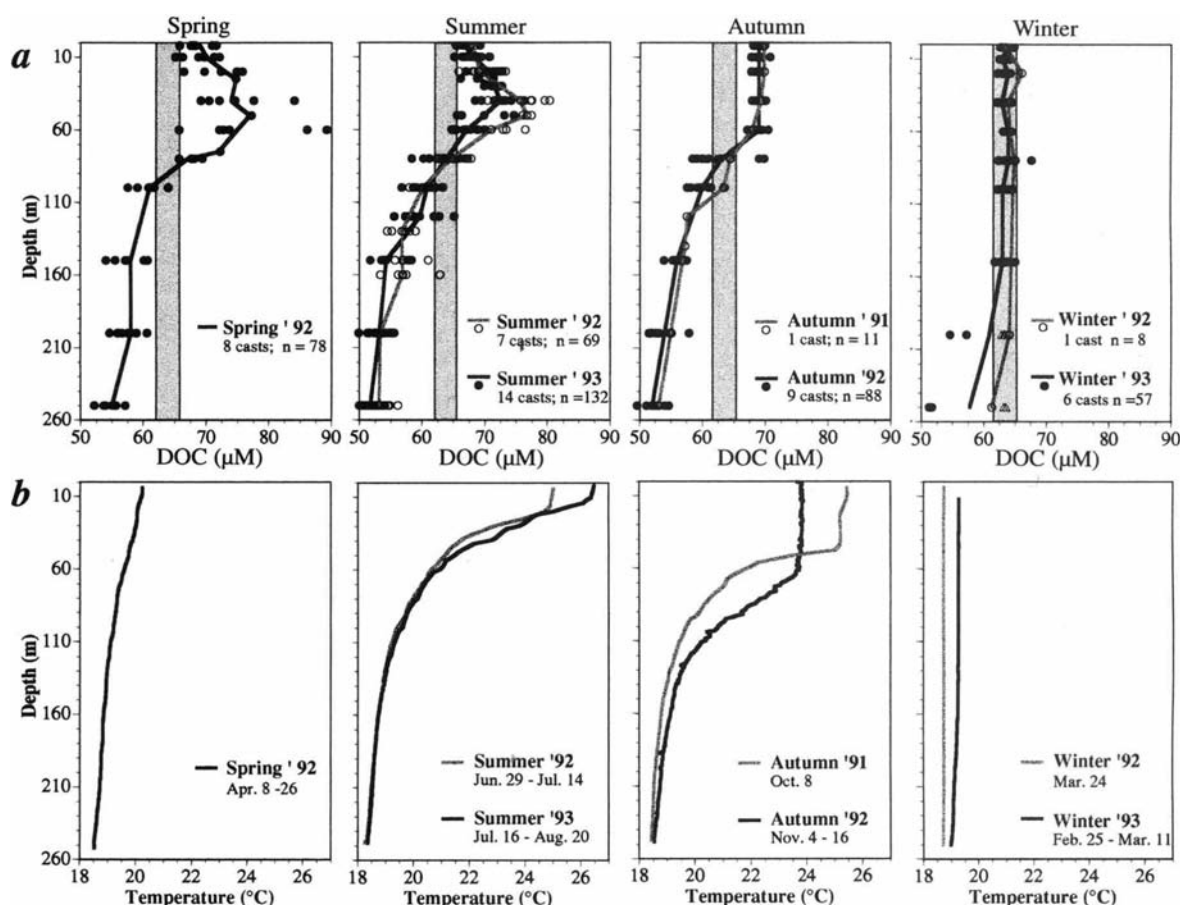


FIG. 1 a, Filled and empty circles are DOC measurements made in the western Sargasso Sea in the vicinity of BATS site ($31^{\circ} 50' \text{ N}$, $64^{\circ} 10' \text{ W}$) and Hydrostation S ($32^{\circ} 10' \text{ N}$, $64^{\circ} 10' \text{ N}$). The lines represent the average profile per season. In winter 1993, the mixed layer extended to 260 m for three casts but samples were only taken in the top 150 m. In all the data we have collected to date, the DOC concentrations were always homogeneously distributed throughout the mixed layer. We assumed this to be true in the three casts in which the whole mixed layer was not sampled. Triangles represent an average of all DOC values within the top 150 m for a given cast and then extended to the base of that cast's mixed layer. In order to emphasize the seasonal dynamics a grey highlighted bar, representing the general range of DOC during winter deep mixing, is shown in each panel. The number of casts and samples (n) collected within a given season are shown in each panel. b, Each panel represents the mean vertical profile of temperature of a given season and year. The dates of the sampling programme are shown in each panel.

METHODS. Niskin bottles were used to collect 10–12 samples per cast within the top 250 m of the water column. The sampling method followed the JGOFS DOC protocol as described previously³¹. Samples were drawn directly from Niskin bottles into precombusted 40-ml vials and sealed with Teflon-coated septa and open top screw caps. Samples were frozen immediately and stored at -20°C until back on land. Before analysis samples were thawed, acidified with 85% H_3PO_4 (30 μl per 30 ml) and sparged with CO_2 -free oxygen at a flow rate of 200 ml min^{-1} for at least 10 min. Samples were analysed by the high temperature combustion technique using a modified Dohrmann DC-190. Operating parameters of the Dohrmann DC-190 were as described previously³¹. All samples were blank-corrected. Analysis was standardized with a 4 point curve of glucose solution in Milli-Q water. Temperature data was collected and processed by Seabird CTD and software for the spring 1992, summer 1992 and winter 1993. Neil Brown CTD was used in the autumn of 1992.

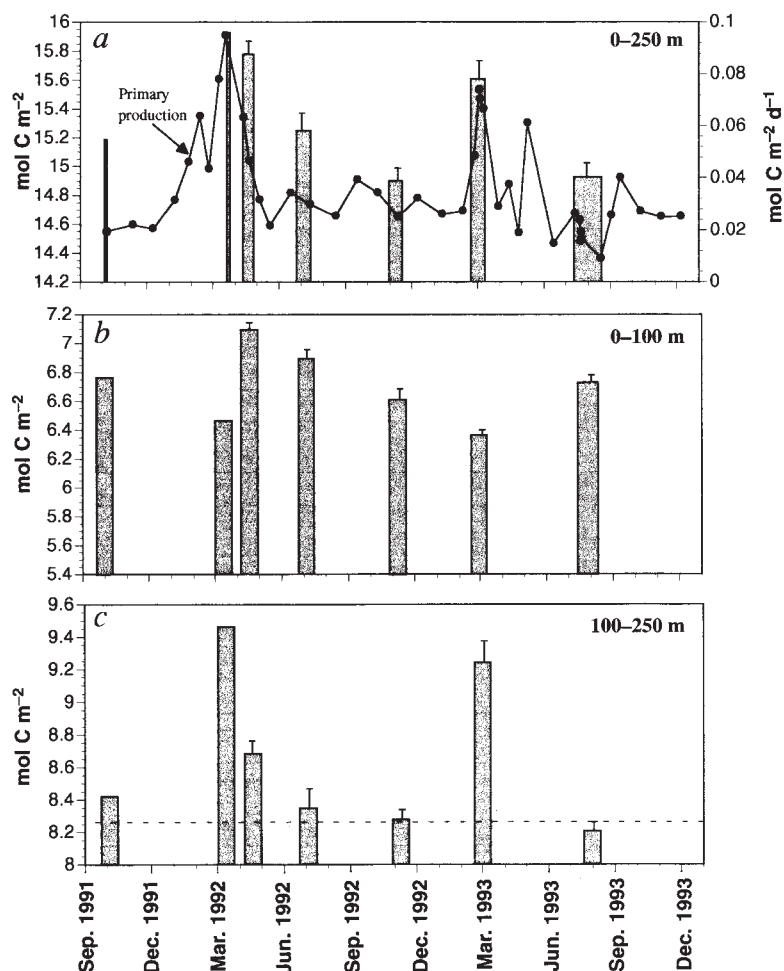


Fig. 2 a, Integrated DOC concentrations and sampling programme (grey bars) compared with the annual pattern of primary production (filled circles). Integrated primary production was estimated by whole day *in situ* ^{14}C incubations in the top 140 m at the BATS site ($31^{\circ} 50' \text{N}$, $64^{\circ} 10' \text{W}$). The width of the bars represents the length of the DOC sampling period. The height of the bars represents integrated DOC concentration in the surface 250 m. The bars in b and c correspond to the average integrated DOC concentration within the 0-100 m and 100-250 m layers respectively. The error bars are for standard error. The broken line in c represents the background integrated DOC value for the 100-250 m layer (see text).

entrainment of low carbon water into the euphotic zone and not by biological consumption alone.

The $\int\text{DOC}$ in the 100-250 m layer increased significantly in both March 1992 and 1993 (Fig. 2c) as a result of entrainment of 'freshly produced' bloom DOC and 'residual' surface DOC. To determine the flux of DOC into the lower layer, we estimated a baseline value of $8.25 \text{ mol C m}^{-2}$ by calculating the mean of all $\int\text{DOC}$ within the 100-250 m layer for autumn 1991, autumn 1992 and summer of 1993 (Fig. 2c). Convection and bloom events resulted in a flux of $\sim 1.21 \text{ mol DOC m}^{-2}$ and $0.99 \text{ mol DOC m}^{-2}$ into the 100-250 m layer in 1992 and 1993 respectively. These flux estimates are approximately 23-42% of Jenkins and co-workers new production estimates, which are equivalent or greater than the estimated annual sinking flux estimates of particulate organic carbon (POC) in waters near Bermuda (Table 1). Upon restratification, the subsurface DOC was trapped, effectively removing it from the euphotic zone. The significant decrease in 100-250 m $\int\text{DOC}$ indicates a subsequent remineralization of this DOC from winter to autumn. Some portion of the exported DOC pool is remineralized rapidly (Fig. 2c; March to April) while another portion representing a semilabile component is mineralized more slowly.

Effective decoupling of DOC production from consumption results in net production in the upper 100 m in spring, and export of DOC escaping mineralization the following winter. This 'residual' pool is comprised of semilabile material left over from the previous year's blooms plus biologically refractory material which has an average age of 4,100 yr (ref. 25). In addition to 'residual' DOC, deep mixing events also export a 'freshly produced' pool. Episodic deep mixing interrupted by short-lived periods of stratification result in production and accumulation

of 'freshly produced' DOC. Upon further deep mixing, as observed in March 1992 and 1993 (Fig. 1), 'freshly produced' DOC, and surface residual DOC are mixed into the 100-250 m layer.

By calculating homogeneous vertical profiles with the same integrated amounts, we determined the potential $\int\text{DOC}$ concentration in each layer resulting from deep mixing. These estimates reflect the residual concentrations resulting from physical mixing in which surface layer DOC was diluted and the deeper layer was enriched. For example, if the $15.2 \text{ mol C m}^{-2}$ in autumn 1991 was redistributed homogeneously over 250 m, then the 0-100 m layer would decrease from 6.8 to 6.1 mol C m^{-2} and the 100-250 m layer would increase from 8.4 to 9.1 mol C m^{-2} . We measured higher values of 6.5 and 9.5 mol C m^{-2} for March 1992 (Fig. 2b, c). We attribute this increase to the production, accumulation and distribution of 'freshly produced' DOC. By comparing the estimated residual $\int\text{DOC}$ in autumn 1991 and 1992 to actual values from March 1992 and 1993 we estimate conservatively that the exported DOC comprised $\sim 32\%$ 'freshly produced' bloom DOC and $\sim 67\%$ 'residual' DOC. Thus, a significant portion of the surface DOC pool was removed from the euphotic zone nearly 1 year after its production.

Our results confirm previous evidence of seasonal DOC changes in marine systems^{10,26} and show a clear seasonal pattern of DOC concentrations and distribution in the surface 250 m of the open ocean. In the northwestern Sargasso Sea, deep mixing events which occur in late February-early March, ventilate nutrient-rich water into the euphotic zone^{6,23,27}. The introduction of these nutrients stimulates a phytoplankton bloom which increases concentrations of particulate and dissolved organic material. Convection mixes DOC-enhanced water below the

euphotic zone. The magnitude and depth of this DOC flux and the size of the seasonal accumulation probably depend on the intensity of the deep mixing event. This export is a mixture of locally produced DOC, DOC produced from the previous spring and refractory DOC. Spring stratification effectively sequesters this organic carbon below the euphotic zone, resulting in net remineralization of DOC within the deep surface layer. Consumption of DOC in this layer suggests that this export is not long-term C storage. Oxygen utilization rates integrated between 100–400 m are an independent estimate of the remineralization of new production⁷ and these agree well with the biogeochemical tracer techniques for determining the total new production near Bermuda (Table 1). However, there are large discrepancies between direct measurements of annual POC flux and the annual export inferred from the geochemical tracers. Michaels and colleagues²⁸ have suggested that sediment traps undersample the sinking particulate flux at the BATS site and this bias could explain a significant portion of this discrepancy. Our data indicate that 23–42% of this discrepancy can be explained by convective mixing of DOC. □

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Increased pressure from rising bubbles as a mechanism for remotely triggered seismicity

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AFTERSHOCKS of large earthquakes tend to occur close to the main rupture zone, and can be used to constrain its dimensions. But following the 1992 Landers earthquake (magnitude $M_w = 7.3$) in southern California, many aftershocks were reported¹ in areas remote from the mainshock. Intriguingly, this remote seismicity occurred in small clusters near active volcanic and geothermal systems. For one of these clusters (Long Valley, about 400 km from the Landers earthquake), crustal deformation associated with the seismic activity was also monitored. Here we argue that advective overpressure^{2–7} provides a viable mechanism for remote seismicity triggered by the Landers earthquake. Both the deformation and seismicity data are consistent with pressure increases owing to gas bubbles rising slowly within a volume of magma. These bubbles may have been shaken loose during the passage of seismic waves generated by the mainshock.

Hill *et al.*¹ describe the seismicity following the Landers earthquake in southern California on 28 June 1992 and show that seismicity levels were higher in a number of regions (all with volcanic history) remote from the Landers mainshock. One is the Long Valley caldera, which has a variety of instrumentation in place because of continuing concern about a possible

eruption⁸. Langbein *et al.*⁸ model ground surface motions during 1989–91 in terms of increased pressure in a spherical source at 7-km depth under the resurgent dome together with growth of a dyke (vertical magma-filled opening) under Mammoth Mountain. These changes resulted in increased seismicity, although the spatial distribution suggests a more complex geometry than in the simple model. Figure 1 shows maps of the Long Valley caldera comparing the seismicity in the 5 days before the Landers earthquake with that in the 5 days after it. Also shown are the sites of a Sacks–Evertson borehole strainmeter⁹ (POP), a long-baseline tiltmeter¹⁰ (LBT) and the proposed pressure sources of Langbein *et al.*⁸. The triggered seismicity has an areal distribution, and magnitudes and depths, similar to those of earthquakes occurring usually in this region¹. The obvious inference is that the triggered seismicity is due to the same process that drives the continuing seismicity, except that after the Landers earthquake the process was enhanced.

Figure 2 shows the cumulative seismicity at Long Valley together with the dilatation at POP and the east–west tilt at LBT (similar plots of seismicity and dilatation were shown in Hill *et al.*¹). We see the clear correlation between the rapid increase in the cumulative number of earthquakes over about 4 or 5 days and the increase in contraction at POP as well as the change in tilt at LBT. Suggested mechanisms for the remote triggered seismicity include hydraulic pumping of high-pressure pore fluids¹, relaxation of partially crystallized magma bodies¹, enhancement by fault connectivity of static strain¹¹ and aseismic slip triggered by dynamic strain^{12,13}. None of these suggested mechanisms can adequately explain either the duration of the triggered activity or the form of the deformation transient. Here, as it seems clear that the continuing seismicity results from increased pressure in magma under Long Valley, we look for an explanation of the triggered seismicity in terms of a process which increases that pressure, resulting in the observed deformations and the increase in seismicity.

We consider the advective overpressure mechanism^{2–5} as a viable candidate. Our attention was drawn to this by Sahagian and Proussevitch^{6,7}, who showed that if a bubble of perfect gas rises in an incompressible liquid inside a rigid sealed container,

the pressure throughout the liquid will increase by ρgh , where ρ is the liquid density and h the height through which the bubble rises. They suggested that this process might be a mechanism for producing increased pressure in a magma chamber; for example, for a 1-km vertical rise, the increase in pressure for the ideal case would be ~ 30 MPa (300 bar). A simple allowance for compressibility of rock and magma ($dV/V = dp/\kappa$, where κ is incompressibility) indicates a pressure increase lower than for the ideal case by a factor of about 3. This factor will depend on the shape of the magma body, but the sparsity of our observations precludes more than qualitative considerations; we restrict our analysis to a feasibility study. Previous work⁸ demonstrates the existence of pressure sources (magma bodies) but does not clearly resolve the geometry of these sources. We note that an increase of a few megapascals in a shallow dyke under Mammoth Mountain would result in the observed dilational stain at POP and roughly one-third of the east–west tilt at LBT (the additional tilt may be due to small pressure increases in the closer, deeper sources); bubbles rising less than 1 km could produce such changes. Such small pressure increases are consistent with a lack of observable changes in the line lengths¹ ($< \sim 1$ mm) monitored (see ref. 8 for locations; MILL sub-net was not occupied) during the days following Landers. Thus, this mechanism passes the first test, that it be capable of producing deformations consistent with the observations.

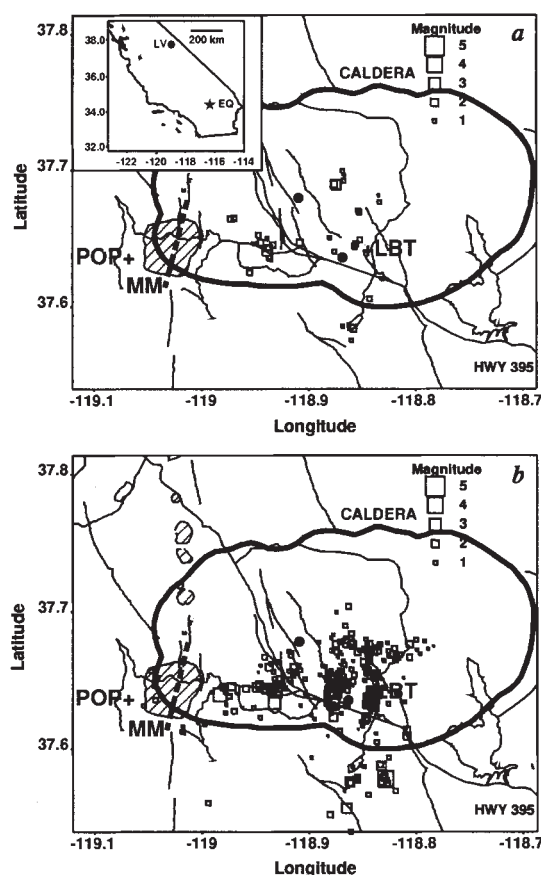


FIG. 1 Map of Long Valley caldera area showing seismicity 5 days before (a) and 5 days after (b) Landers. The insert map in a shows part of California and the locations of Long Valley (LV) as a solid circle and of the Landers earthquake (EQ) as a star. The caldera maps show the locations of the borehole strainmeter (POP) and long-baseline tiltmeter (LBT). Solid circles and heavy dashed line indicate the locations³ of the magma deep pressure source and dyke, respectively; the two deep source locations were determined for different time intervals. The Inyo Domes are shown with diagonal hatching; MM, Mammoth Mountain. The seismicity patterns are suggestive of a magma source with complex geometry.

Next we consider how this process could be initiated. The deformation changes began when the seismic waves generated by Landers passed through the area. Perhaps during the rarefaction stages of the waves, the pressure in the magma is lowered sufficiently for dissolved gas to form bubbles throughout the magma. We reject this as a significant source as such a process would result in a relatively large and instantaneous increase in pressure; such a pressure increase would be observable as a rapid change in dilatation at POP (such rapid tilt at LBT would not necessarily be detected because of acceleration effects of local microseismic activity). More likely is the release of pre-existing bubbles that are held down by surface tension effects. These bubbles will exist because as magma previously rose from greater depths through a series of narrow conduits (cracks), the pressure decreased and dissolved gases formed small bubbles^{14–16}. Entry into the magma body is presumably through a highly fractured region in which these small bubbles can be captured at the abundant solid surfaces. Bubbles that are marginally held down (vertical force balance) may readily be shaken loose by the movement of the magma induced by the seismic waves, because Landers was a source of high-amplitude, long-period waves¹. An indication of the size of such bubbles can be obtained by considering the vertical force balance on a bubble adhering to a flat horizontal surface. This is a balance between the upthrust due to buoyancy and the downward component of the surface tension forces. The net vertical downward force may be written $F = 2\pi\gamma r \sin^2\alpha - 2\pi\rho g r^3(2\cos^3\alpha + 3\cos^2\alpha - 1)/6$, where γ is the surface tension, α the half-angle of the cone subtended at the

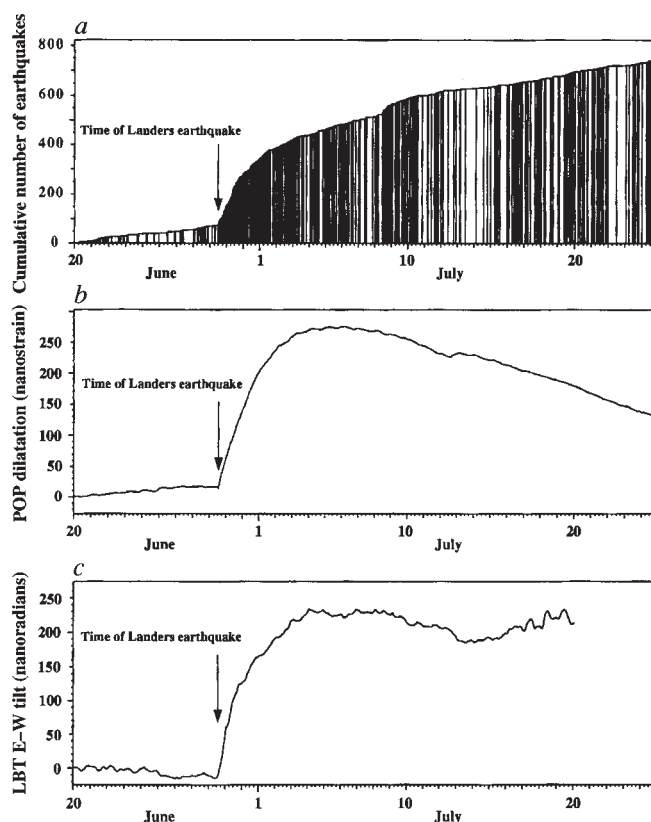


FIG. 2 a, Cumulative number of earthquakes in the Long Valley caldera area; b, dilatation (contraction positive) at POP; c, east–west tilt at LBT. The rapid increase in the rate of earthquake occurrence corresponds closely to the increase in contraction and tilt. North–south tilt has a similar shape to the east–west tilt, but with lower amplitude. Seasonal strain changes at POP preclude knowledge of the extent of recovery at POP; initial slow tilt-recovery seems to have been interrupted on 12 July by a nearby deformation event which also shows as a small positive excursion on POP.

centre of the bubble by the circle of contact, r is the radius of the bubble and ρ is the density contrast between magma and gas. The first term is due to surface tension and the second is the Archimedean upthrust on a truncated spherical bubble (for $\alpha=0$, it reduces to the standard expression for a wholly immersed spherical bubble). This second term gives an upward force for small α and is zero for $\alpha=60^\circ$. For greater values of α , a bubble of any radius will experience a net downward force. Magma densities and surface tensions do not depend strongly on the magma chemistry¹⁷, so the balance condition is primarily a function of r and α . Bubbles with radius ~ 5 mm will be marginally stable for small $\alpha \sim 5^\circ$.

The increases in contraction at POP and in the seismicity occur over an interval of ~ 4 d. Thus, for the advective overpressure mechanism to be significant the bubbles must be able to rise vertically less than 1 km during that time. For our purposes, Stokes' law may be written $r^2 = 9h\eta/2gt\rho$, where r is the radius of a bubble that will rise through a vertical height h in time t for a liquid of viscosity η and density contrast ρ . Figure 3 shows, as a function of viscosity¹¹, the size of bubble that will rise 1 km in 4 days. Magma composition in the area is likely to be either basaltic or rhyolitic¹⁸. For a basaltic magma we see that bubbles ~ 1 cm in diameter released from near the bottom of the chamber would be able to increase the pressure on a timescale which matches the change in strain and tilt and the increase in seismicity rate. As indicated above, bubbles of that size would be held down marginally with very small angles of contact. The period of roughly 4 days during which contraction increases is then a natural consequence of the ascent velocity of sub-centimetre-size bubbles. Smaller bubbles rising as aggregates (with higher velocities than isolated bubbles¹⁹) would also be consistent with such timescales. That the strain rate decreases during this time is also reasonable: there is likely to be a size distribution of bubbles; larger ones will get to the top sooner and then the pressure increase rate will decrease; the effective viscosity higher in the chamber may well be higher (leading to slower ascent velocities) because of lower temperature and/or partial crystallization.

The initial rapid (4 d) increase in seismicity is followed by a return to the background rate over an interval of a few tens of days. Whether or not this was accompanied by deformation recovery is unclear. The tilt record (Fig. 2) does not show significant recovery; the dilatation record has been adjusted by removal of an estimated seasonal change and the uncertainty in this procedure precludes reliable knowledge of dilatation recovery. After the Landers earthquake there was an increase in

$^3\text{He}/^4\text{He}$ in gas discharged from a fumarole near Middle Mountain²⁰ which would be consistent with increased loss of gas from the magma reservoir. Even if there is no recovery in deformation, we expect that the seismicity rate would return to the earlier value as this is characteristic of aftershock activity, which decreases with time although coseismic static deformations persist.

We have shown here that advective overpressure due to rising gas bubbles is a viable model to explain the remote triggered seismicity in the Long Valley area following the Landers earthquake. In contrast to previous suggestions^{1,10-12}, the time variations of the seismicity and deformations are a simple consequence of the mechanism producing the excess magma pressure. Many of the other sites of triggered seismicity may have magma chambers¹ and we suggest that this mechanism could be the general explanation for the triggered seismicity following the Landers earthquake. $\square$

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Deep bacterial biosphere in Pacific Ocean sediments

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ALTHOUGH around 70% of the Earth's surface is marine, little is known about the microbiology of underlying sediments, which can be more than a kilometre deep¹. Selective degradation of organic matter within sediments over geological time profoundly affects the chemical composition of the ocean and atmosphere². Microbial processes have a fundamental role in surface sediments^{3,4}, but despite geochemical evidence⁵, their significance in deeper sediments has not been established⁶. Here we report the discovery of viable sediment bacterial populations at five Pacific Ocean sites to depths >500 m. Bacterial distributions and activities are commensurate with geochemical changes. Bacterial profiles with depth are remarkably consistent, and deviations can be linked to specific environmental factors. The rate of decline in these populations indicates that bacteria are present to even greater depths.

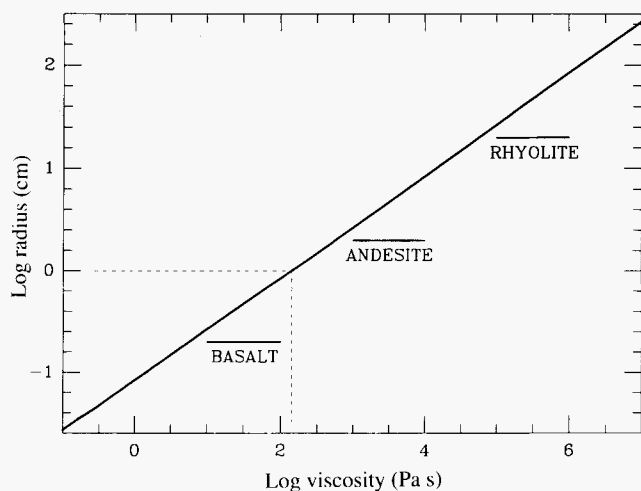


FIG. 3 Plot based on Stokes' law showing size of bubble that would rise 1 km in 4 days as a function of viscosity. For a basaltic magma, such bubbles would have a size of a few millimetres (dashed lines).

These bacteria, some of which are unique, must have a profound effect on deep-sediment diagenetic processes, and their presence considerably extends the biosphere.

Pacific Ocean sediments were collected on five Ocean Drilling Programme Cruises (ODP Legs 112, 128, 135, 138, 139) and a Charles Darwin Cruise (Leg 38). Deepest sediments were obtained in 900 m water depth in the Japan Sea (Leg 128) and using a microscopic technique to detect bacteria directly in the sediment⁷, intact bacterial cells were found to be present even in the deepest sample, 518 m below sea floor (mbsf, Fig. 1). There is an initial rapid and significant ($P < 0.05$) decline in this total bacterial population from a surface maximum of 1.4×10^9 cells cm^{-3} to 3.0×10^7 cells cm^{-3} at 6 mbsf. Below this, bacterial populations decline more slowly, with occasional increases which persist over considerable depths. The population at 518 mbsf (1.1×10^7 cells cm^{-3}) represents a significant ($P < 0.01$) 120-fold decrease from the near surface value, but remains substantial.

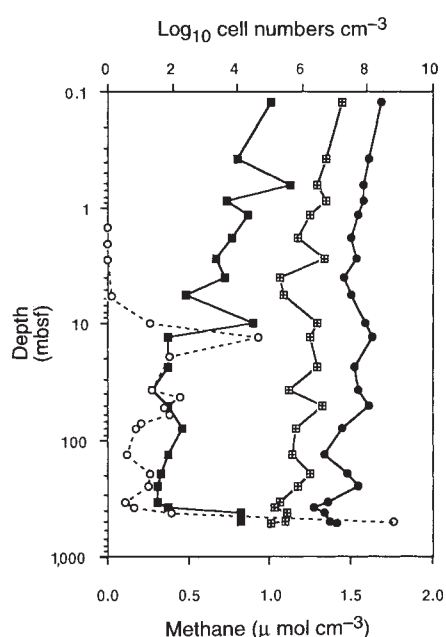


FIG. 1 Depth profiles of bacteria and pore water methane from the Japan Sea sediments. Total count (●), dividing and divided cell count (■), total viable anaerobic heterotrophic bacteria (□), pore water methane concentration (○). Modified from ref. 9. Comparison of gradients indicated no significant difference between total and dividing cell profiles.

METHODS. Whole round cores (WRC, 25 cm) were cut with a sterile hacksaw blade from the middle of 1.5-m core sections, which had been wiped with alcohol and placed in a specially constructed sterile cutting rig⁹ flushed with sterile oxygen-free nitrogen (OFN). The cut ends were then flamed and capped with sterile core caps while gassing with sterile OFN. These cores were then transported and stored anaerobically²¹ at 4 °C before analysis a few weeks later. All laboratory handling was conducted at 16 °C (mean down core temperature) under a flow of sterile OFN using aseptic techniques in a laminar flow cabinet. Intact sub-cores (5 cm³) for further analysis were taken vertically with sterile, modified, 5 ml plastic syringes and sealed with sterile 'Suba Seals'. Sediment adjacent to the core liner was not sampled. Replicate sub-samples were taken at 5 cm intervals for direct bacterial analyses and stored in filter-sterilized (0.2 μm) 4% formaldehyde in artificial sea water before analysis^{7,9}. Data represent the mean for each 25 cm WRC. Identical analysis of samples taken on board ship from the cut ends of cores before capping showed no overall significant difference from the transported samples (total count $P = 0.11$, dividing cells $P = 0.40$, $n = 21$). Total viable anaerobic heterotrophic bacteria was the sum of all most-probable-number (MPN) anaerobic viable bacteria (heterotrophs, nitrate reducers, sulphate reducers, ammonifiers, acetogens⁹). Pore water methane concentrations are derived from ref. 12.

Overall, the depth distribution of dividing and divided bacterial cells is similar to, and a constant percentage of, the total population (Fig. 1, ~4.8%). The presence of dividing cells indicates that a proportion of the total bacterial population is active and viable, as dividing cells correlate with an independent measure of bacterial productivity ([H³]methyl thymidine incorporation) in deep sediments⁸. This was confirmed by the presence of culturable bacteria (Figs 1, 2)⁹, the rapid growth of bacteria in enrichment slurries¹⁰ and the presence of high molecular weight DNA¹¹, indicative of intact bacteria, even in the deepest layers. The profile of anaerobic heterotrophic bacteria is similar to the total population, although more exaggerated, especially the significant increase ($P < 0.01$) below ~360 mbsf, which coincides with a large increase in thermogenic C₁–C₅ hydrocarbons¹². As there is no other geochemical change in this region it may be that a portion of the bacterial population used this new energy source. Although sulphate-reducing bacteria (SRB) able to grow on methane have not been isolated, they have been implicated in the anaerobic oxidation of methane¹³, and there is a small but sustained increase in rates of sulphate reduction below about 360 mbsf (Fig. 2) coinciding with an increase in reduced sulphur species⁹.

Depth distribution of bacterial activity (Fig. 2) is consistent with changes in the bacterial population and with corresponding geochemical changes. For example, sulphate-reduction rates correlate significantly ($P < 0.05$) with changes in pore water sulphate concentration (Fig. 2) and the concentration of reduced sulphur species⁹. These data also correlate ($P < 0.002$) with the presence of viable SRB (Fig. 2), indicating that a meaningful subset of the natural population was isolated. Sulphate reduction is the dominant terminal oxidizing process⁹ and hence SRB

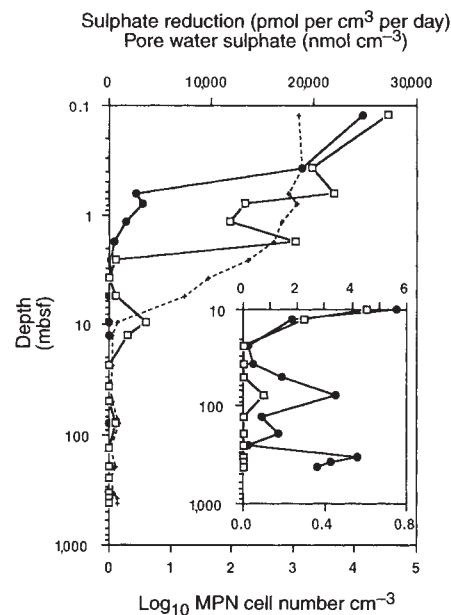


FIG. 2 Depth profiles of bacterial sulphate reduction rates (●), viable SRB (□), and pore water sulphate concentration (+) from Japan Sea sediments. Inset, scale-expanded section of the lower portion of the main graph and has the same axis units. Modified from ref. 9.

METHODS. Rates of sulphate reduction were determined in 5-cm³ sub-cores, incubated at mean down-hole temperature (16 °C), from the proportion of ³⁵S-labelled sulphide produced from an injection of 7.2 μl (=2.28 μCi) of ³⁵SO₄²⁻ (ref. 22). Triplicate subcores were incubated anaerobically for three different incubation times, which increased with deeper samples. These combined results minus a time zero blank were used to calculate rates. MPN²³ SRB were enumerated on Postgates' medium, with either lactate or acetate as growth substrate²⁴. Pore water sulphate concentrations were calculated from ref. 12.

should have characteristics reflecting those of the general bacterial community. Pure cultures of SRB from 80 mbsf enrichments are *Desulfovibrio* species which, on the basis of 16S rRNA gene sequence analysis, show most homology with *Desulfovibrio salexigens* (88% similarity, over 785 base pairs). In terms of metabolism (13 out of 33 metabolic tests different), salt tolerance, temperature and pressure characteristics the isolates are, however, very different from *D. salexigens*. Our isolates are barophilic and active up to at least 27.5 MPa, in contrast to the type strains of superficially similar SRB, which are not active above 15 MPa (Fig. 3). The maximum sulphide production for strain 80-55 is at 10 MPa, the calculated *in situ* pressure. The very broad and high range of growth temperatures for the Japan isolates (15 to 65 °C, with a slight optimum at 25 °C) is completely unlike those of known SRB (Fig. 3). The temperature and pressure characteristics of these new isolates would allow them to be active well in excess of 1,000 mbsf in the Japan Sea.

Direct 16S rRNA gene sequence analysis of the sediment¹⁴ (1.6, 9.8, 78 and 503 mbsf) shows the presence of a diverse bacterial community, including SRB, some members of which seem to represent novel lineages of bacteria¹⁵.

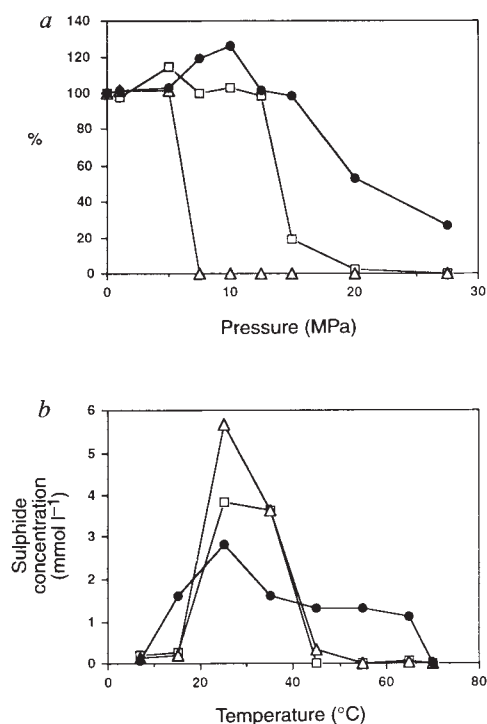


FIG. 3 Effect of elevated incubation pressure (a) and temperature (b) on the growth (as reflected by sulphide production) of Japan Sea isolate, strain 80-55 (●) and type strains *D. salexigens* DSM 2638 (△) and *D. desulfuricans* DSM 642 (□). The amount of sulphide produced by active cultures of SRB at the given pressure is expressed as a percentage of the sulphide produced by control cultures grown at atmospheric pressure (0.1 MPa). Cultures were grown under hydrostatic pressure at 25 °C, until turbidity was observable in the control cultures (~5 days) at the same temperature. Temperature profile samples were cultured until maximum turbidity was reached before sulphide determinations were made²⁵. All cultures were grown under strict anaerobic conditions using medium described²⁶ and purity was checked by lack of growth on complex media and 16S rRNA gene sequence analysis¹⁴. Sulphide production for strain 80-55 at 10 MPa is significantly greater ($P < 0.02$) than for type strains at the same pressure. The temperature characteristics of the Japan Sea isolates were reproducible and were significantly different ($P < 0.001$) from the type strains, which had a maximum growth temperature of approximately 40 °C.

Sediment disturbance and contamination problems are common to all sediment studies, but the coring and sampling procedures of ODP consistently produce reliable and meaningful data. In addition, for our results, the consistency between data from different microbiological measurements and those from geochemical analyses is indicative of negligible contamination. The increases in bacterial populations and activities in the deeper layers cannot be explained by contamination from smearing during coring, as these increases were preceded by regions (>100 m) of low populations and activity. It was evident from good core recovery (99%) and all shipboard measurements that coring disturbance was minimal and individual horizons were excellently preserved¹².

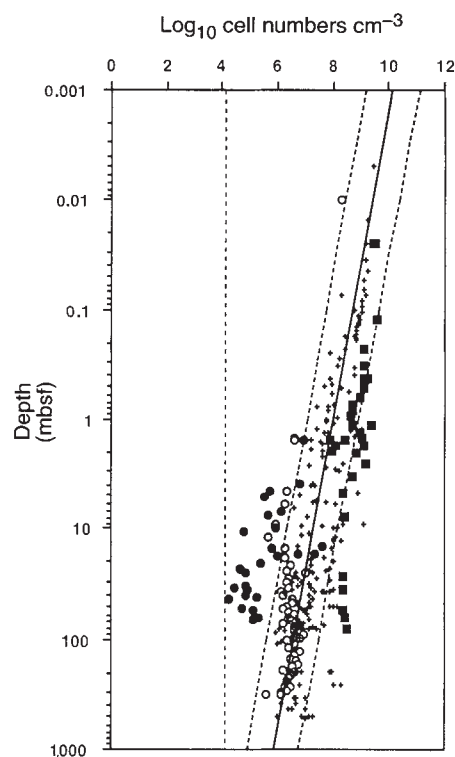


FIG. 4 Depth distribution of total bacterial numbers from five geographically distinct sites in the Pacific Ocean. Data presented ($N=299$) represent means of triplicate counts. A regression line is fitted ($\log_{10}$ count = $8.06 - 0.715 \log_{10}$ depth (m), $r^2 = 0.49$) and 95% prediction limits (based on triplicate counts) have been constructed. The vertical dashed line represents the minimum direct count detection limit calculated when counting samples containing the lowest bacterial populations. Three sites are highlighted. Shallow Peru Margin (■, ODP Leg 112²⁷, Darwin Leg 38, very high productivity) bacterial concentrations are consistently greater than the regression line, and many lie outside the upper prediction limit. Eastern equatorial Pacific (○, ODP Leg 138²⁸, very low productivity) bacterial concentrations lie below the regression line in the upper layers of the sediment. The Pliocene/Pleistocene boundary at this site, estimated at 30 mbsf²⁸, represented a transition to high organic carbon sediments in deeper layers and this is reflected in bacterial concentrations approaching the regression line below 30 mbsf. Juan de Fuca Ridge (●, ODP Leg 139²⁹, hydrothermal) bacterial concentrations are, with the exception of two data points, below the regression line, and the majority of data occur outside the lower prediction limit. Other data (+) are a combination of Japan Sea (ODP Leg 128¹²), Lau Basin (ODP Leg 135³⁰), Juan de Fuca Ridge non-hydrothermal site (ODP Leg 139²⁹) and deep Peru Margin sites (ODP Leg 112²⁷). Ranges of sedimentary conditions at these sites were: pH 7–8, temperature 3–80 °C, organic carbon 0.04–4%, ammonium 10–12,000 $\mu\text{mol l}^{-1}$ and phosphate 0–240 $\mu\text{mol l}^{-1}$. The oldest sediment containing bacteria was approximately 10 Myr (Leg 135³⁰).

Bacterial population profiles in deep sediment layers at the four geographically distinct locations in the Pacific Ocean are remarkably similar to those in the Japan Sea (Fig. 4) and likewise decrease significantly with sediment depth ($P < 0.001$). Some deviations from the trend occur which can be linked to specific environmental factors. Elevated bacterial populations occur in a shallow water, high productivity Peru Margin site. Conversely, reduced populations are present in low productivity (Eastern Equatorial Pacific) and hydrothermally heated sites (Juan de Fuca Ridge). There is no indication that the rate of decrease in bacterial populations will change in deeper sediments, and hence it is likely that bacterial populations are present to much greater depths. Temperature is a limiting factor, but with a thermal gradient of $30\text{--}100\text{ }^{\circ}\text{C km}^{-1}$ in sediments and a probable upper temperature for bacterial growth of $110\text{--}150\text{ }^{\circ}\text{C}$ (ref. 16), it would only be limiting in very deep or hydrothermal sediments. A depth integration of bacterial numbers to only 500 mbsf (average oceanic sediment depth¹) produces, however, a considerable total bacterial biomass of $\sim 1.5\text{ t ha}^{-1}$ organic carbon (average bacterial volume = $0.21\text{ }\mu\text{m}^3$, $310\text{ fgC }\mu\text{m}^{-3}$ (ref. 7), density = 1). Although this only constitutes a small percentage of global sedimentary organic carbon (0.004%), remarkably it is 10% of the living carbon in the surface biosphere¹⁷.

These findings significantly extend the depth of the marine biosphere and indicate that sedimentary organic matter, including molecular fossils¹⁸, will undergo continued bacterial modification long after burial. The deep biosphere also responds to, and modifies, geochemical fluxes (for example, CH_4 ; Fig. 1) analogous to, but probably more significant globally than, biological interactions at hydrothermal vents and in oil reservoirs¹⁹. The presence of viable bacterial populations in deep marine sediments is consistent with recent results from other deep subsurface environments such as aquifers²⁰. □

Role for supplementary motor area cells in planning several movements ahead

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To achieve a volitional goal, we need to execute multiple movements in a specific temporal order. After repetitive performance of a particular sequence of movements, we are able to memorize and execute the whole sequence without external guidance. Where and how in the brain do we store information necessary for the orderly performance of multiple movements? We have found a group of cells in the cerebral cortex of monkeys whose activity is exclusively related to a sequence of multiple movements performed in a particular order. Such cellular activity exists in the supplementary motor area^{1,2}, but not in the primary motor cortex^{3,4}. We propose that these cells contribute a signal about the order of forthcoming multiple movements, and are useful for planning and coding of several movements ahead.

We trained two monkeys (*Macaca fuscata*) to perform three movements (push, pull or turn a manipulandum) in four different orders. Before each movement, monkeys waited for a tone signal that served as a movement trigger. After completion of an individual movement, a mechanical device returned the manipulandum to a neutral position within which the animal had to hold the manipulandum and wait for a next trigger signal. Initially, during the learning phase, green, yellow and red lights indicated that the correct movement required was to push, pull or turn the manipulandum, respectively. The animals received five learning trials and subsequently performed the sequential motor task in the absence of the visual cues. The success rate was over 95% in all motor sequences. Electromyographic analysis showed that the forelimb muscles were active for only a short period during the execution of individual movement, but not active during the period when animals were waiting for the next movement trigger signal.

The classically defined supplementary motor area (SMA) is now divided into two areas^{2,5,6}: the rostrally situated presupplementary area (pre-SMA) or F6, and the caudally situated SMA proper (or F3). We have recorded from both areas delineated by the criteria reported previously⁶. This report deals with 206 cells recorded in the caudal part (for the sake of brevity, called simply the SMA). Of these, 54 cells were of interest in that they were preferentially active in relation to a particular order of forthcoming movements guided by memory. Although these cells were active during a waiting period before the first movement trigger signal, the activity increased only if the order of forthcoming three movements was a specific one. The activity of this type of SMA cell is shown in Fig. 1; for instance, it was active while the animal was waiting to perform a motor sequence of turn–pull–push (Fig. 1, bottom). It is unlikely that the activity of this cell signals a preparatory process for the initial movement in the sequence because the cell was not active when the animal was ready to perform a different sequence of turn–push–pull (Fig. 1, top). In addition, the cell was not active when the animal performed the three movements in other sequences (e.g. push–pull–turn). Furthermore, the cell was not active if the three movements were guided by a visual signal, even if the order was turn–pull–push. These cells then seem to signal a specific order of forthcoming, multiple movements to be performed on the basis of memory.

We found another group of 74 cells in the SMA that were preferentially active during the interval between two specific movements. These cells were active during a waiting period

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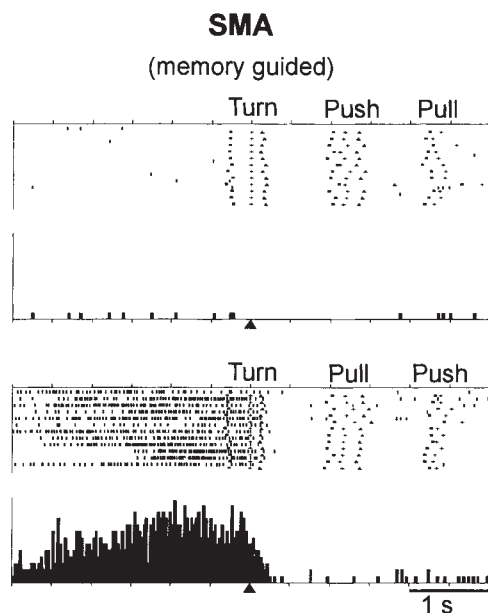


FIG. 1 Activity of a cell in the SMA exhibiting preferential relation to a specific order of three movements performed without sensory guidance. This cell is active during a waiting period, but only if the sequence of upcoming movements is in the order of turn, pull and push (bottom). In raster displays, each row represents a trial, and dots represent individual discharges of this cell. Small squares, crosses, and triangles denote the times of occurrence of the trigger signal, movement onset, and target acquisition. In histograms, discharges over 12 trials are summed. Triangles at the bottom indicate the start of the first movement. METHODS. Standard electrophysiological techniques for single-cell recording were used²⁷. Monkeys, sitting in a primate chair, were required to place a manipulandum to a neutral position and wait 2.5–4.5 s for the first movement trigger signal (high-pitch tone). When the animal performed the first movement, a mechanical device returned the manipulandum to the neutral position. While keeping the manipulandum in this position, the animal had to wait about 1 s for the second, and then for the third movement trigger signal. A series of three correct movements was rewarded with delivery of apple sauce, 500 ms later. The average time interval between motor sequences was 7 s. Initially, the correct movement was indicated with green, yellow and red lights. During this learning period of visually guided five trials, the animal had to learn the correct sequence, after which the sequential motor task was performed on the basis of memory. After completion of six trials of the memorized sequential task, random flashing of lights (for 2 s) signalled the end of current sequence, and the beginning of new sequence.

before execution of a particular movement, but only after the performance of another particular movement. An example of this type of cell is shown in Fig. 2. This cell was active during the waiting period preceding a forthcoming pull movement and following the performance of push movement (Fig. 2, top, SEQ1 and SEQ3). The same cell was not active during the waiting period before a pull movement if the previous movement was to turn (Fig. 2, bottom; SEQ2 and SEQ4). These cells seem to signal a temporal linkage combining two specific movements.

We also analysed cellular activity from the primary motor cortex (MI, $n = 106$) in the same animals, and found that properties of cellular activity were different from those in the SMA. A great majority (90%) of cells in MI was active in close temporal association with the execution of a particular movement or movements. Figure 3 shows a typical example of such movement-related cellular activity in MI. In this cell, the activity started shortly before, and lasted during execution of push movement. As demonstrated, the temporal order of the individual movements within a sequence did not influence the magnitudes

of the activity changes. A smaller number of MI cells ($n = 27$) exhibited activity during the waiting periods. These cells were active while the animals were waiting to perform a particular movement, regardless of the temporal sequence of the three movements.

Previous studies have reported that cells in the SMA are active in close time relation with movement execution^{7–10}, supporting a view that the SMA takes some part in execution of movements, even when the motor task is simple^{11–13}. In line with these previous reports, such movement-related activity was also found in 25 cells in the present study (an example is shown in Fig. 4). On the other hand, it has also been reported that the SMA has a more significant role when motor tasks are more demanding^{14–21}. Clinical findings in human subjects with brain lesions including the SMA have found that the difficulties among patients are most pronounced in relation to sequential movements or sequential performance of multiple movements^{22,23}. Monkeys with SMA lesions also showed abnormalities in sequential motor tasks^{4,24}. Such motor deficits become more

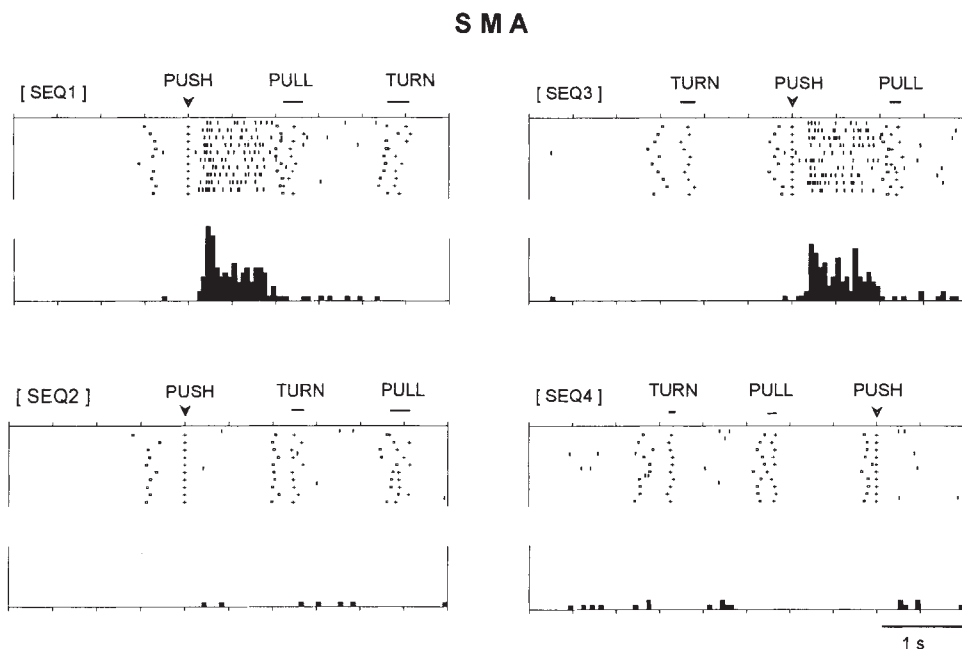


FIG. 2 Selective activity of an SMA cell during a waiting period in between a single combination of two specific movements. This cell is active before the 'pull' movement if the previous movement is 'push' (top, SEQ1 and SEQ3) but not if the previous movement is 'turn' (bottom).

M I

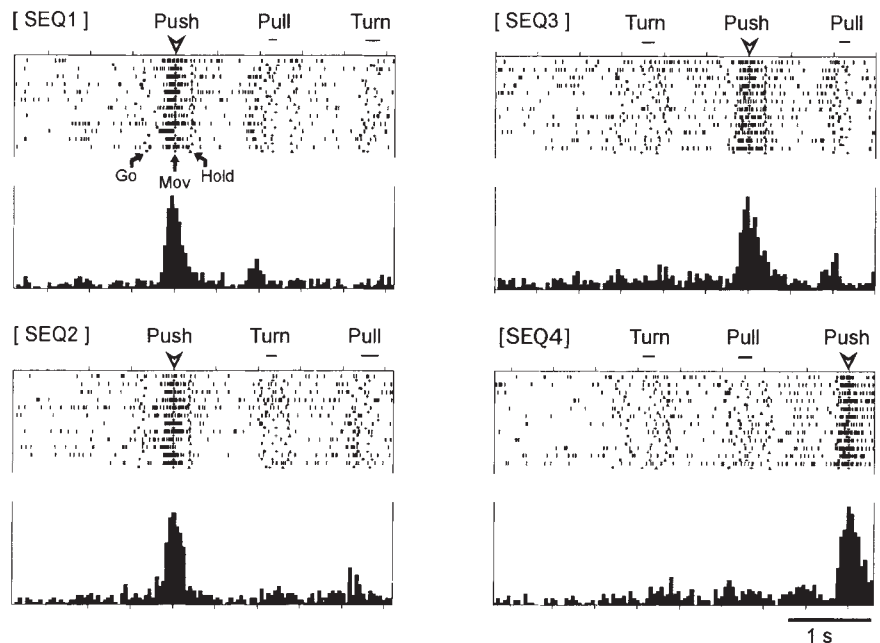


FIG. 3 Activity of a movement-related cell commonly observed in MI. This cell is active during a short period of execution of 'push' movement. The magnitude of activity is not influenced by the temporal order of the three movements.

S M A

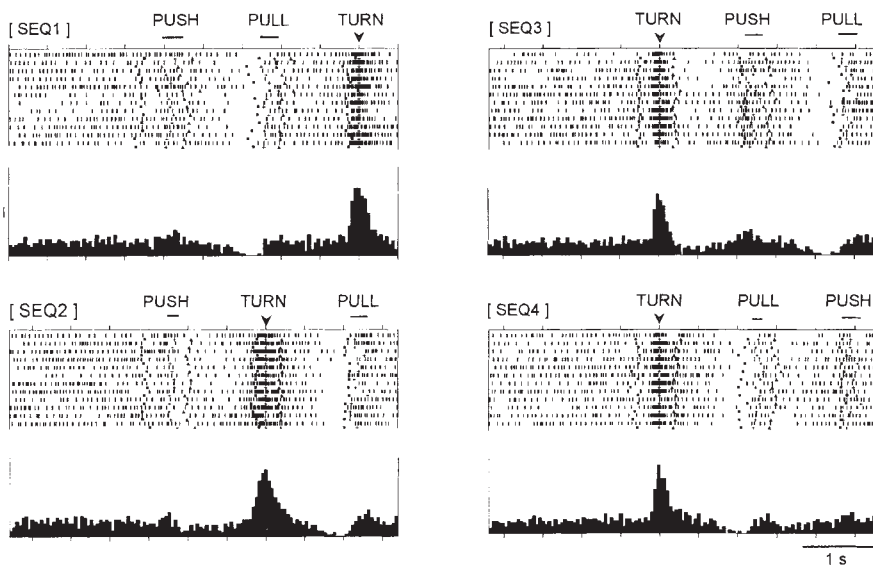


FIG. 4 Activity of an SMA cell that is categorized as movement-related. This cell is active shortly before and during 'turn' movement.

apparent when subjects had to perform the motor task on the basis of memory²⁵. The abundance of the preparatory type of activity in waiting periods in this study is in accord with these reports, and indicate that cellular activity in the SMA seems different depending on the nature of the motor task required.

Our results lend support to a hypothesis that the SMA is crucial for temporal structuring of movements. The cellular activity in two different cortical areas suggest that the SMA, but not the MI, is profoundly involved in performing multiple movements in a predetermined order. The order-specific cellular activity, as shown in Fig. 1, could provide a signal about the order of forthcoming movements, useful for programming temporal structure of an intended motor act that includes multiple movements. On the other hand, the SMA cells that are specifically active during an interval between a particular combination of two movements in a specific order (as shown in Fig. 2) are

useful for coding a particular time sequence of two movements. We propose that these two classes of cellular activity provide a means by which the SMA is involved in temporal sequencing of multiple movements. It is important to note that the role of the pre-SMA in motor preparation or structuring forthcoming motor action has been implicated in previous reports^{6,26}. We will describe elsewhere the cellular activity in the pre-SMA during performance of the same motor task as in the present study. □

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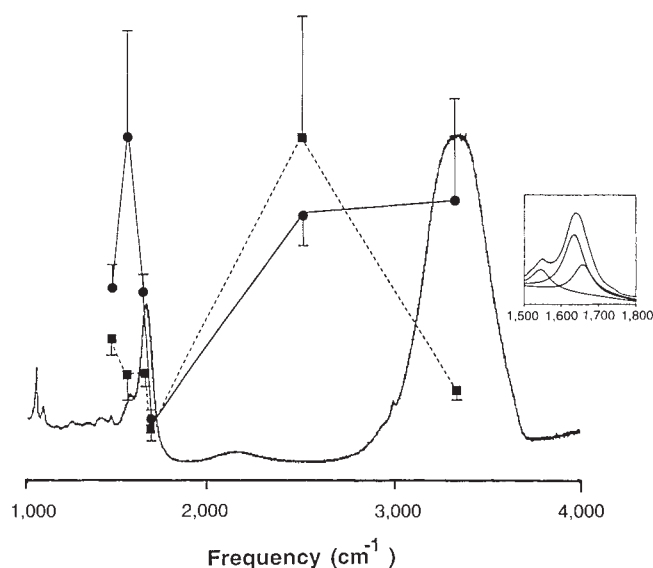


FIG. 1 Relative ablation yield (●) and collateral damage (■) plotted against the absorption spectrum for corneal stroma.

METHODS. Spectra were taken as previously described¹³. The dominant mid-infrared feature was the OH-stretch mode of water near $3,300\text{ cm}^{-1}$ ($3,000\text{ nm}$) and, as such, is the most efficient wavelength for absorbing infrared (IR) radiation in the $2,000\text{--}10,000\text{ nm}$ range. The partially resolved feature centred near $1,640\text{ cm}^{-1}$ ($6,100\text{ nm}$) comprises the bending mode of water near $1,640\text{ cm}^{-1}$, the amide II mode near $1,550\text{ cm}^{-1}$ ($6,450\text{ nm}$) and the amide I mode near $1,665\text{ cm}^{-1}$ ($6,000\text{ nm}$); curve fitting results are shown in the insert. IR wavelengths between $1,500\text{--}1,700\text{ cm}^{-1}$ ($5,900\text{--}6,600\text{ nm}$) will excite both water and proteins through these overlapping modes. At a wavelength of $6,450\text{ nm}$ the extinction lengths of water and protein are $12\text{ }\mu\text{m}$ and $8.5\text{ }\mu\text{m}$ respectively, corresponding to more than 100 stromal layers. Both the ablated volume and the thickness of collateral damage were estimated from histology. Relative values were scaled to the spectrum for presentation; for comparison the mean value for collateral damage was $12\text{ }\mu\text{m}$ ($6,000\text{ nm}$, $13\text{ mJ per macropulse}$) and the mean value for ablation yield was $2.5 \times 10^5\text{ }\mu\text{m}^3\text{ mJ}^{-1}$ ($6,450\text{ nm}$). These data are for exposure to single FEL macropulses at $3,000$, $4,000$, $6,000$, $6,100$, $6,450$ and $6,850\text{ nm}$. Ablation was also investigated as a function of wavelength, macropulse energy, number of macropulses, and repetition rate of the macropulses (not shown).

Tissue ablation by a free-electron laser tuned to the amide II band

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EFFORTS to ablate soft tissue with conventional lasers have been limited by collateral damage and by concern over potential photochemical effects^{1–5}. Motivated by the thermal-confinement model⁶, past infrared investigations targeted the OH-stretch mode of water with fast pulses from lasers emitting near $3,000\text{ nm}$ (refs 1, 7–9). What does a free-electron laser offer for the investigation of tissue ablation? Operating at non-photochemical single-photon energies, these infrared sources can produce trains of picosecond pulses tunable to the vibrational modes of proteins, lipids and/or water. We report here that targeting free-electron laser radiation to the amide II band of proteins leads to tissue ablation characterized by minimal collateral damage while maintaining a substantial ablation rate. To account for these observations we propose a novel ablation mechanism based on compromising tissue through resonant denaturation of structural proteins.

Laser ablation aims to achieve tissue removal by 'cold' etching, that is, where the etch pattern is defined by the irradiating beam and peripheral tissue is free from thermal (collateral) damage. Free-electron lasers (FELs) are broadly tunable, pulsed sources providing both high average and high peak power^{10,11} and great versatility for applications research^{12–17}. The Vanderbilt FEL (ref. 18) operates in the $2,000\text{--}10,000\text{ nm}$ region, enabling a comparison of tissue ablation at $3,000\text{ nm}$ with other infrared wavelengths that were previously unavailable. It emits trains of roughly picosecond micropulses, separated by 350 ps

with $\sim 17,000$ micropulses per macropulse, generating up to 30 macropulses per second. To guide our selection of exposure wavelengths we measured the absorption spectra of a range of different types of tissues: sclera and especially the cornea are highly organized, collagenous tissues; dermis additionally contains proteoglycans and elastin in a more complex fibrillar architecture; brain is a non-collagenous, cellular tissue. Ocular and neural tissues have about the same water content, whereas dermal tissue is less hydrated. Despite these distinctions, the infrared spectrum for cornea (Fig. 1) is typical of soft tissue¹³. Figures 2–4 compare ablation of the various tissues at $6,450$ and $3,000\text{ nm}$. Figure 1 also summarizes the wavelength dependence for cornea, demonstrating remarkable ablative properties for wavelengths near $6,450\text{ nm}$. Similar wavelength dependencies were obtained in other tissues, with further reduction in collateral damage for neural tissue at $6,450\text{ nm}$ (Fig. 3a).

FEL peak powers were in the megawatt range, raising the possibility of nonlinear or multiphoton effects. With regard to the former, the anharmonic shift (270 cm^{-1}) of the OH-stretch mode of water, as measured with picosecond pulses¹⁹, is a fraction of spectral widths ($\sim 400\text{ cm}^{-1}$) as measured here. As for the latter, the rate of tissue removal did not exhibit the power-law dependencies indicative of multiphoton processes²⁰.

There are two popular models for infrared laser ablation. The optical-breakdown model is based on plasma and subsequent shock wave formation, cavitation and tissue disruption^{1,7}. Breakdown thresholds have been determined for two pulse durations of Nd:YAG radiation: 1 TW cm^{-2} for 40-ps pulses and 0.3 TW cm^{-2} for 10-ns pulses⁷. We approached these threshold values; however, at the intensities reported here we did not observe plasma formation²¹ in either water or soft tissue. Furthermore, a model solely based on optical breakdown would not account for our experimental observations. The thermal-confinement model⁶ recognizes competing thermal effects: the vaporization of water driving an explosive ablation versus thermal diffusion leading to collateral damage. It accounts for the observation^{8,9} that collateral damage is limited if the pulse duration is less than the thermal relaxation time of the ablated volume. The thermal-confinement model predicts that 3,000 nm radiation will be the most efficient infrared wavelength for ablating tissue, which is not supported by the experimental observations presented here. Thus we propose an alternative model that recognizes the importance for infrared tissue ablation of both compromising the mechanical integrity of tissue and developing a pressure head through the liquid-vapour transition. We first applied this partitioning-of-energy model to corneal stroma²². Infrared radiation in the 5,900–6,600 nm range will be absorbed by both proteins and water. After the infrared absorption, there is a brief moment when both species have nonequilibrium energy distributions. What mechanisms govern this ablative process? Pyrolytic fragmentation of biopolymers occurs at temperatures ranging from 400–1,000 °C and the activation energy for chain scission or depolymerization is 900–1,400 J g⁻¹ (ref. 23). At

lower energies, however, collagen undergoes structural transitions from highly ordered arrays of crosslinked triple helices with high tensile strength to amorphous gelatin, a far less resilient material^{24,25}. The specific heats of native and denatured collagen are 1.35 and 1.55 J per gK (ref. 26), respectively; the peak transition temperature is near 60 °C, and the enthalpy of denaturation is $\sim 55 \text{ J g}^{-1}$ (ref. 27). Thus the 'melting' of collagen to gelatin will be energetically more accessible than either vaporization ($2,260 \text{ J g}^{-1}$) or pyrolytic fragmentation, indicating that the protein absorption leads to loss of structural integrity whereas the water absorption provides the explosive force as accounted for by the thermal confinement model. Thermal confinement occurs for micropulse but not macropulse durations, providing a plausible explanation for the collateral damage observed with 3,000 nm FEL irradiation as compared with corneal ablation using conventional infrared lasers^{7,9}; this suggests that selectively reducing the macropulse duration may further limit collateral damage. Clearly, stroma lends itself to biophysical modelling; however, the wavelength dependence for the ablation of soft tissues were similar, suggesting a common mechanism based on neither tissue organization nor collagen content *per se*, but instead on the selective denaturation of structural proteins. It is straightforward to generalize the partitioning-of-energy model to account for the ablation of ocular, neural and dermal tissues. Furthermore, additional studies of neural tissue (unreported data) show a comparable pattern of ablation in animals killed two weeks after irradiation, in contrast to the extensive region of necrosis that develops post-exposure during survival studies using the 'cw' carbon dioxide laser⁴.

FIG. 2 Histological sections summarizing FEL ablation of cadaver ocular tissue. The optimal wavelength, as determined by ablation yield and collateral damage, for ablating ocular tissue was 6,450 nm. Sclera: a, 6,450 nm radiation, macropulse energy 16 mJ. The ablated volume (incision) was 1,060 μm deep and 260 μm wide. The average thickness of collateral damage was 40 μm . b, 3,000 nm radiation, macropulse energy 30 mJ. Section of a collapsed, full-thickness ablation canal; collateral damage had an average thickness of 300 μm . In a and b, 100 macropulses at 4 Hz were received. Cornea: c, 6,450 nm radiation, macropulse energy 20 mJ. The ablated volume was 260 μm wide and 350 μm deep. The thickness of collateral damage was 40 μm or less. d, 3,000 nm radiation, macropulse energy 30 mJ. The ablated volume was 200 μm wide and 190 μm deep. The average thickness of collateral damage was 150 μm . c and d received 10 macropulses. For macropulse energies between 1–2 mJ corneal ablation was typically accompanied by persistent collateral damage $\sim 10\text{-}\mu\text{m}$ thick. Collateral damage increased at higher macropulse energies. At higher energies and for larger numbers of macropulses, lacunae or voids were observed near the periphery of the collateral damage.

METHODS. Human ocular tissue was provided by the Nashville Lions Eye Bank. Partial separation of tissue is a mounting artefact. Original magnification for all frames in the figures was $\times 100$, except for c, which was $\times 50$. About 2,600 sections were fixed, mounted, and stained with haematoxylin and eosin (ocular and neural tissues) or trichrome (dermis) during this investigation. See Fig. 3 legend for details of exposure conditions.

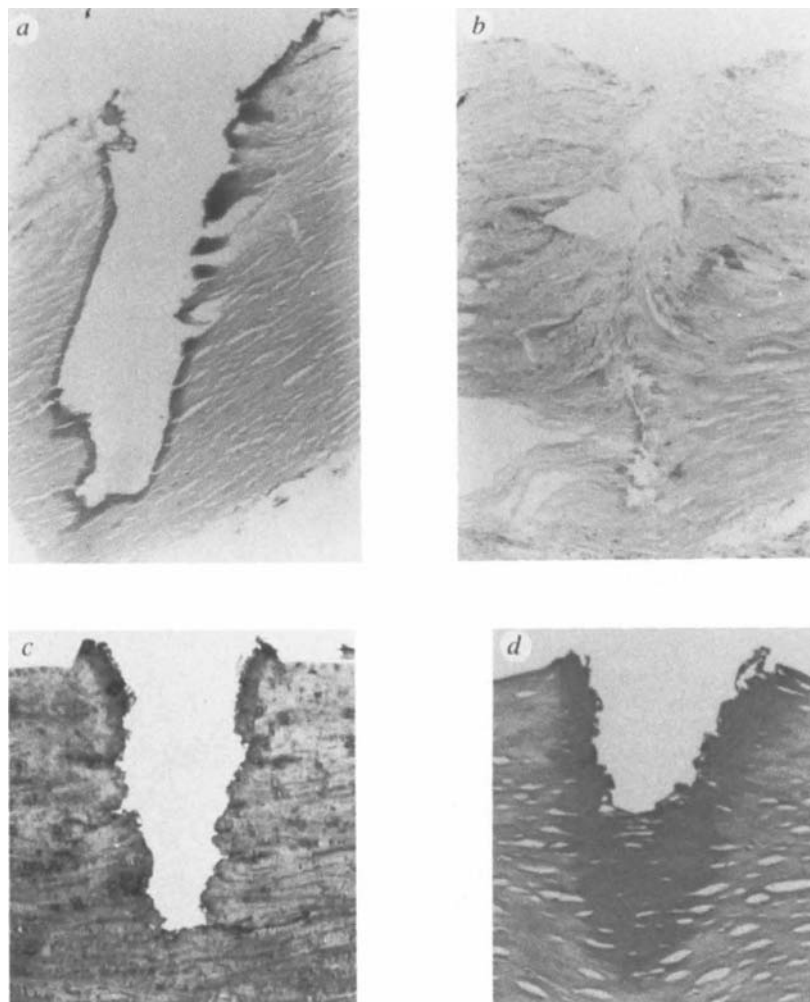


FIG. 3 Histological sections summarizing FEL ablation of rat brain cortex. The optimal wavelength for ablating neural tissue, as determined by ablation yield and collateral damage, was 6,450 nm. *a*, 6,450 nm radiation, macropulse energy 20 mJ. The total depth was 3,060 μ m. Note the absence of perceptible collateral damage (coagulation necrosis) laterally. At the deep aspect of the ablation collateral damage measures $\sim$ 4 μ m. Bottom fold was a mounting artefact. *b*, 2,940 nm radiation, macropulse energy 20 mJ. The total depth was 3,500 μ m. Note the considerable amount of collateral damage between the surface and the trough of the ablated volume (incision), as indicated by loss of cellular markings. Inserts have an additional magnification of $\times$ 17. Both *a* and *b* received 100 macropulses at 4 Hz.

METHODS. Sprague-Dawley rats (200 g) were used for neural investigations: protocols for this and other experiments were approved by the Institutional Animal Use Committee. Each brain was dissected, irradiated, and fixed (see Fig. 2 legend) within 2 hours of death. The FEL beam was delivered in 1, 10, 100 and 1,000 macropulse static exposures, depending on the tissue, for ocular or neural tissue and repeatedly scanned in the continuous mode (20 Hz repetition rate) for dermis. The FEL was tuned to 3,000, 4,000, 6,000, 6,100, 6,270, 6,450 and 6,850 nm with a bandwidth of 30 cm^{-1} ; the micropulse duration was 1.0 ± 0.5 ps. The theoretical diameter of the focused beam was $\sim$ 100 μ m; however, histology may be a better estimate given the extensive scattering of infrared radiation by tissue.

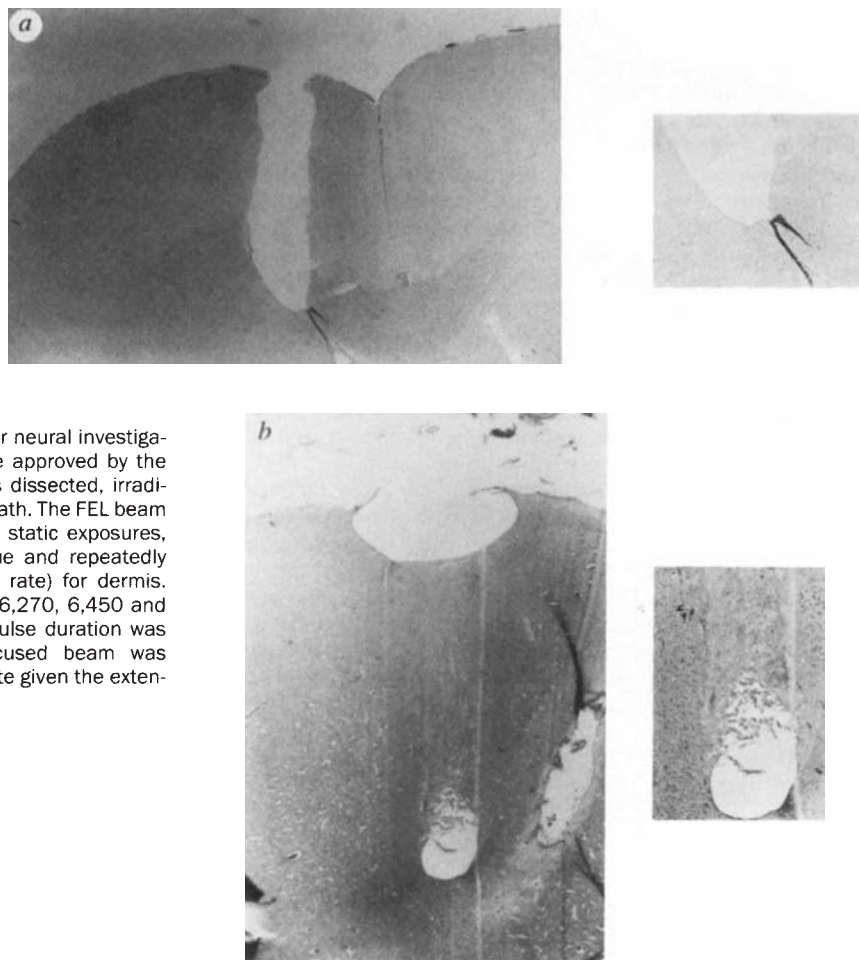
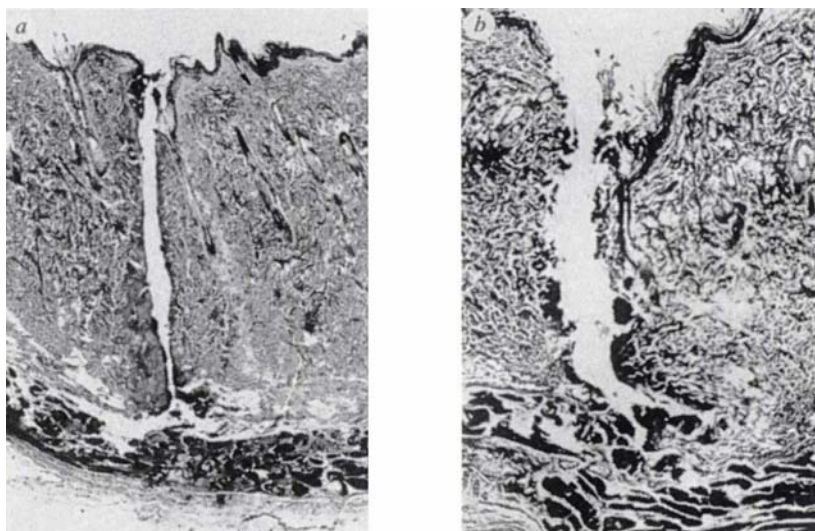


FIG. 4 Histological sections summarizing FEL ablation of rat dermis. Macroscopic ablation of dermis was achieved with repetitive scans of the FEL beam: 6,000, 6,100, 6,270 and 6,450 nm irradiation was accompanied by markedly less collateral damage relative to 6,850 and 3,000 nm and especially 2,500 and 4,000 nm. Thus the optimal wavelengths for ablating dermis, as determined by collateral damage alone, were in the 6,000–6,450 nm range. *a*, 6,450 nm radiation, macropulse energy 20 mJ; *b*, 3,000 nm radiation, macropulse energy 30 mJ.

METHODS. Sprague-Dawley rats (200 g) were anaesthetized intraperitoneally with Ketaset (ketamine hydrochloride, 22.0 mg per kg body weight) and Rompun (xylazine, 2.2 mg per kg body weight). With the onset of analgesia, the dorsal pelt of each animal was clipped, shaved and the FEL beam focused on the dorsum. Lateral incisions that extended to the panniculus carnosus were made at each wavelength by repetitive scans in the continuous mode. Incisions (3 cm) were immediately sutured after exposure. The animals were killed within 4 h of incision. See legends of Figs 2 and 3 for additional details.



In summary, we have shown the efficacy of ablating tissue by targeting energy to the amide modes. While the investigation of ocular and neural tissues indicates the precision of the ablation process, dermis highlights the capability for macroscopic incisions. This mechanism should generalize to many tissues with obvious surgical and clinical implications. □

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p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5

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CYCLIN-dependent kinase 5 (Cdk5) was originally isolated through its structural homology to human Cdc2¹, a key regulator of cell-cycle progression²⁻⁶. In tissue samples from adult mice, Cdk5 protein is found at the highest level in brain, at an intermediate level in testis, and at low or undetectable levels in all other tissues, but brain is the only tissue that shows Cdk5 histone H1 kinase activity⁷. No equivalent kinase activity has been found in tissue culture cell lines despite high levels of Cdk5. This raised the possibility that a Cdk5 regulatory subunit was responsible for the activation of Cdk5 in brain. Here we describe the cloning and characterization of a regulatory subunit for Cdk5 known as p35. p35 displays a neuronal cell-specific pattern of expression, it associates physically with Cdk5 *in vivo* and activates the Cdk5 kinase. p35 differs from the mammalian cyclins and thus represents a new type of regulatory subunit for cyclin-dependent kinase activity.

Cdk5 kinase activity correlates with the extent of differentiation of neuronal cells in the developing mouse neocortex⁷. To study the activation of the Cdk5 kinase in a system more amenable to biochemical analysis, primary cultures were prepared from gestational-day-18 (E18) rat embryonic cerebral cortices. Neuronal cells in these cultures undergo terminal differentiation within ~7 days, during which a constant level of Cdk5 protein was detected (Fig. 1a), whereas Cdk5 kinase activity increased in parallel with the increasingly differentiated phenotype of the cultured cells (Fig. 1b).

Activation of the kinase activity of known cyclin-dependent kinases requires a positively acting regulatory subunit known as

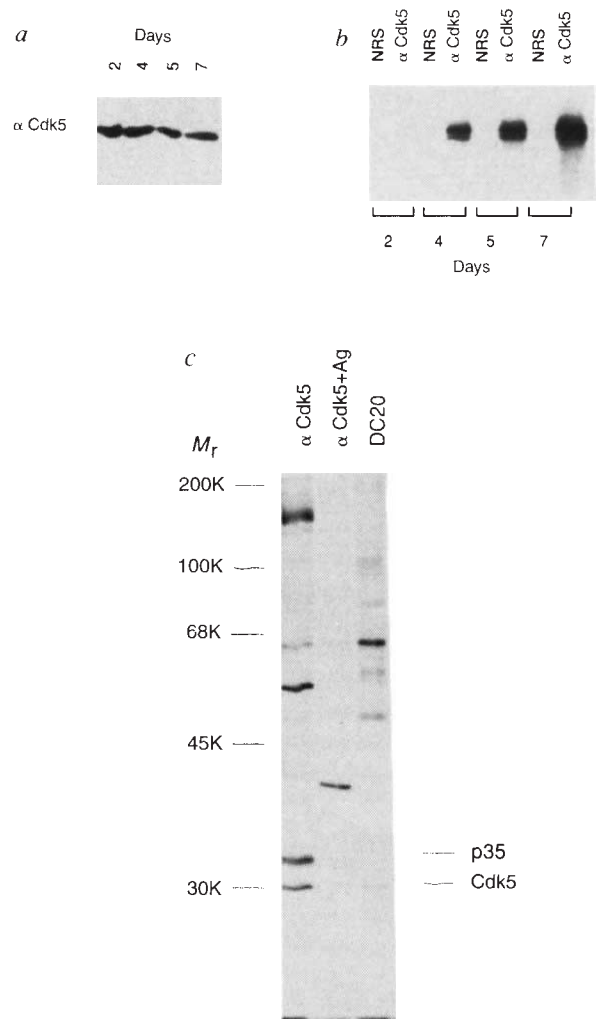


FIG. 1 Expression and activity of Cdk5 in cultures derived from E18 rat embryonic forebrain. *a*, Constant level of Cdk5 protein expression in 2-day, 4-day, 5-day and 7-day cultures was detected by a monoclonal antibody to Cdk5 (DC17). *b*, Temporal pattern of Cdk5 kinase activity in 2-day, 4-day, 5-day and 7-day cultures was detected with PA1 antibody⁷ using histone H1 as *in vitro* substrate. Rabbit preimmune serum (NRS) was used as negative control. *c*, 7-day primary neuron cultures were metabolically labelled with [³⁵S]methionine, lysed, and proteins immunoprecipitated with PA1, or PA1 preincubated with the peptide antigen, or DC20 (a monoclonal antibody to Cdk5). In addition to Cdk5, 35K, 60K and 180K proteins were coprecipitated with PA1. These additional protein bands were abolished by pre-incubating the antibody with the cognate antigen. As this antibody only recognized Cdk5 in western blot analyses and in denaturing immunoprecipitation experiments⁷, the 35K, 60K, and 180K proteins seemed to be precipitated by virtue of their association with Cdk5.

METHODS. Primary rat neuronal cultures were made from the cerebral cortices of the E18 fetal brains according to ref. 13. At 2, 4, 5 and 7 days after plating, cells were collected and lysed in lysis buffer⁷. *a*, For western blotting, 20 µg total protein was loaded onto each lane. After electrophoresis, proteins were transferred to Immobilon-P membrane (Millipore). The blot was then probed with DC17 (mouse monoclonal antibody to Cdk5; L.-H.T. unpublished results), followed by incubation with horseradish peroxidase-labelled secondary antibody to mouse IgG and detection with the ECL developing system (Amersham). *b*, For *in vitro* kinase assays, cell lysates collected on different dates were immunoprecipitated with rabbit preimmune serum and PA1. Kinase reactions were performed as described¹⁴. Histone H1 (2 µg) was used as exogenous substrate. *c*, For immunoprecipitation from metabolically labelled cells, 7-day neuronal cultures were labelled with 200 µCi ml⁻¹ [³⁵S]methionine (NEN) for 4 h. Cell lysates were immunoprecipitated with PA1, or PA1 preincubated with Cdk5 C-terminal peptide, or DC20 (L.-H.T., unpublished results).

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a cyclin⁸. To test for the existence of a Cdk5 regulatory subunit, 7-day-old cultures of rat neuronal cells were metabolically labelled with [³⁵S]methionine and immunoprecipitated with anti-Cdk5 antibodies. Figure 1c shows that proteins of relative molecular mass 35,000, 60,000 and 180,000 (*M_r* 35K, 60K, 180K) were coprecipitated with a Cdk5-specific antibody PA1⁷. Examination of the 35K protein showed that it was a regulatory subunit for Cdk5 (the nature and origin of the 60K and 180K proteins are still unclear). A 35K phosphoprotein was

coprecipitated with Cdk5 from lysates of cells labelled with ³²P-inorganic phosphate (data not shown), and a 35K band became heavily phosphorylated when Cdk5 immune complexes were tested in an *in vitro* kinase assay in the absence of exogenously added substrate (data not shown). These experiments suggest that the Cdk5-associated 35K molecule is a phosphoprotein and a potential substrate for the Cdk5 kinase.

Previously, Lew *et al.*⁹ purified Cdk5 holoenzyme from bovine brain and detected a 25K protein in the purest fractions of active

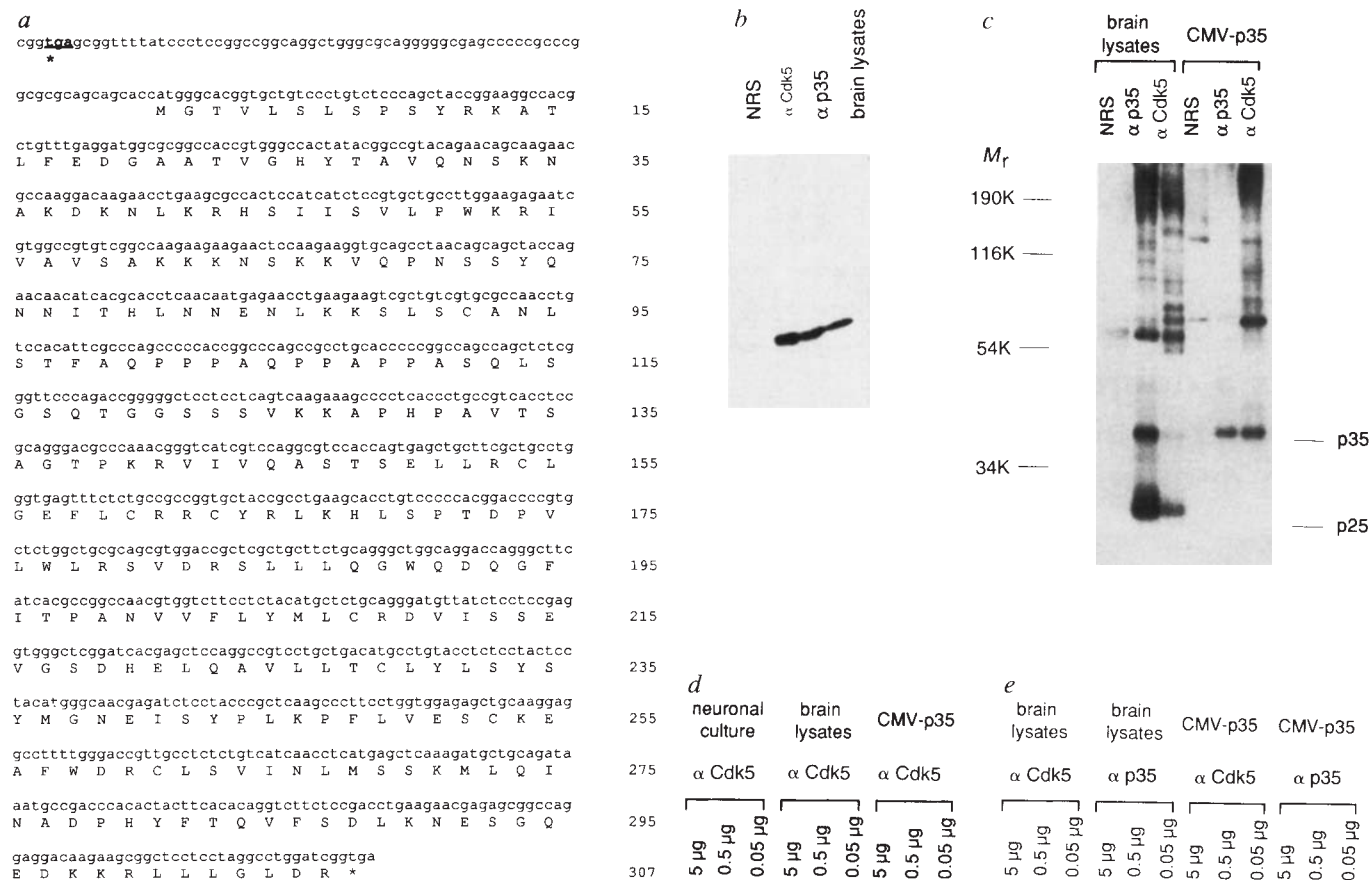


FIG. 2 Cloning of p35 and association of p35 with Cdk5. **a**, Nucleotide and amino-acid sequences of the entire human p35 cDNA open reading frame. The GenBank accession number of human p35 is X80343. **b**, Cdk5 detected in western blot analysis of immunoprecipitates prepared from bovine brain lysates with antibodies to p35 or Cdk5. The filter was probed for Cdk5. Total bovine brain lysates (5 μg) were used as control. **c**, p35 immunoprecipitates prepared from bovine brain lysates and CMV-p35-transfected cells contain an associated kinase activity. The 35K protein phosphorylated by the kinase that comigrated with the Cdk5-phosphorylated 35K protein is indicated. The p35-associated kinase also recognized the 25K protein that was phosphorylated by Cdk5 kinase activity from bovine brain lysates. **d**, Comparison of the partial proteolytic digestion patterns of 35K proteins. The 35K protein phosphorylated by Cdk5 from primary neuronal culture, the 35K protein phosphorylated by Cdk5 from bovine brain lysates, and phosphorylated p35 from CMV-p35-transfected cells were digested with *S. aureus* V8 protease. The identical digestion patterns indicate that p35 encodes the 35K proteins associated with Cdk5 in primary neuronal culture and in brain lysates. **e**, The V8 proteolytic map of the 35K protein immunoprecipitated by the anti-p35 antibody from brain lysates is identical to those of Cdk5-associated and phosphorylated 35K protein and the recombinant p35 recognized by its own antibody from CMV-p35 transfected cells.

METHODS. **a**, PCR primers corresponding to amino-acid residues PPASQL to TDPVLW were used to generate a DNA fragment from total bovine brain RNA using peptide information available from J. Lew and J. Wang¹⁰. This DNA fragment was used to screen a human fetal brain cDNA library (Stratagene). Twelve positive clones were isolated after

screening 1×10^6 phage, two of which (4.0 kb and 4.1 kb) seemed to contain the p35 full-length opening reading frame. **b**, For immunoprecipitations and western blotting, 2 mg bovine brain lysates were immunoprecipitated with rabbit preimmune serum, PA1, and rabbit anti-p35 antiserum. Blotting and detection procedures are described in Fig. 1a. DC17 was used to probe the blot for the presence of Cdk5. Antibody to p35 was generated by immunizing rabbits with bacterially produced $6 \times$ histidine-tagged p35 (see Fig. 3 legend). **d** and **e**, Proteolytic mapping with *S. aureus* V8 protease¹⁵ was performed as described⁷.

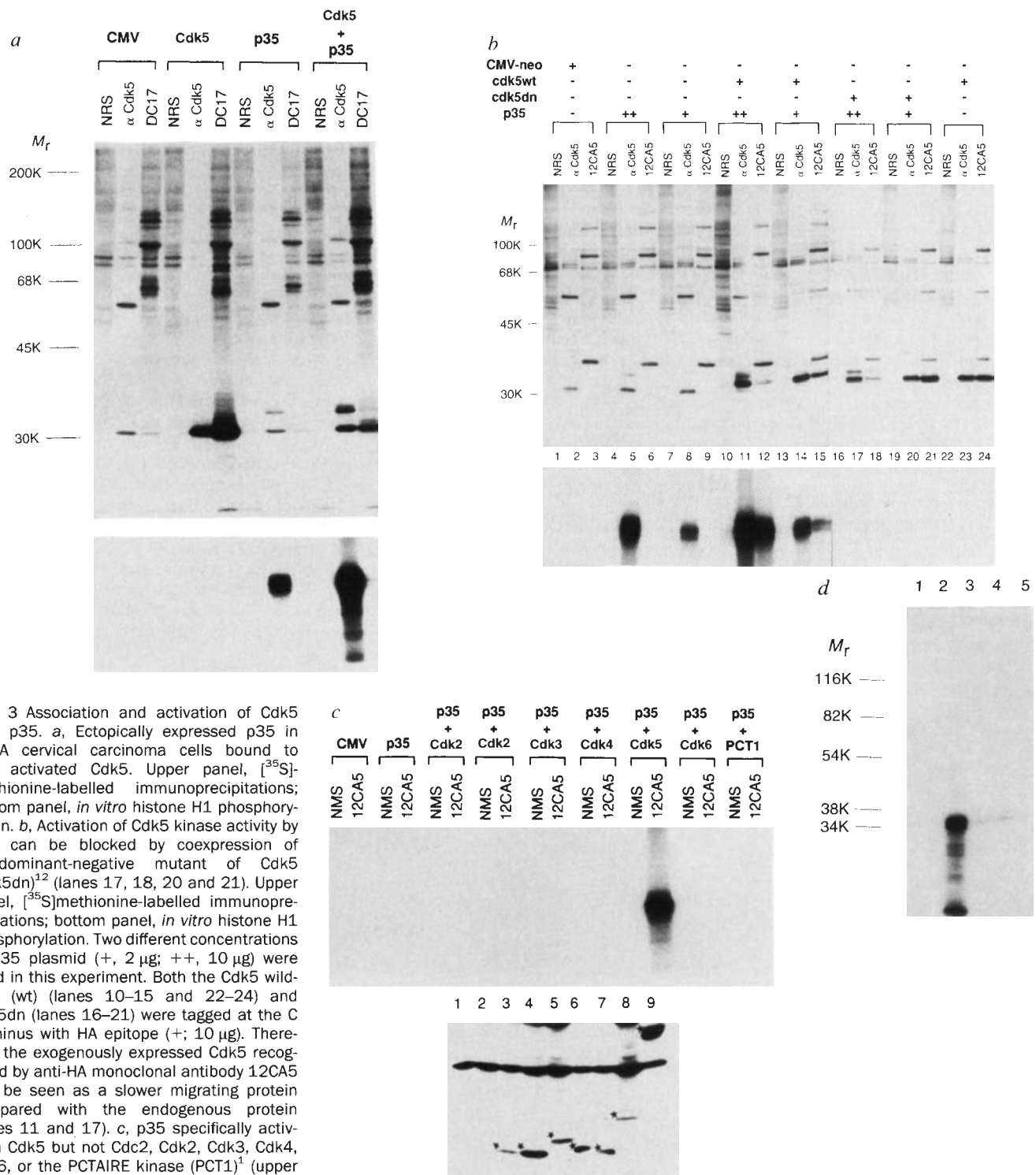


FIG. 3 Association and activation of Cdk5 with p35. **a**, Ectopically expressed p35 in C33A cervical carcinoma cells bound to and activated Cdk5. Upper panel, [35 S]-methionine-labelled immunoprecipitations; bottom panel, *in vitro* histone H1 phosphorylation. **b**, Activation of Cdk5 kinase activity by p35 can be blocked by coexpression of a dominant-negative mutant of Cdk5 (Cdk5dn)¹² (lanes 17, 18, 20 and 21). Upper panel, [35 S]-methionine-labelled immunoprecipitations; bottom panel, *in vitro* histone H1 phosphorylation. Two different concentrations of p35 plasmid (+, 2 μ g; ++, 10 μ g) were used in this experiment. Both the Cdk5 wild-type (wt) (lanes 10–15 and 22–24) and Cdk5dn (lanes 16–21) were tagged at the C terminus with HA epitope (+; 10 μ g). Therefore the exogenously expressed Cdk5 recognized by anti-HA monoclonal antibody 12CA5 can be seen as a slower migrating protein compared with the endogenous protein (lanes 11 and 17). **c**, p35 specifically activated Cdk5 but not Cdc2, Cdk2, Cdk3, Cdk4, Cdk6, or the PCTAIRE kinase (PCT1)¹ (upper panel). Each Cdk was HA-tagged at its C terminus. Expression of the kinases in transfected C33A cell lysates was confirmed by western blotting using 12CA5 (lower panel). Asterisks indicate protein bands corresponding to different Cdk5. Lane 1, CMV-neo; lane 2, CMV-p35; lane 3, CMV-Cdc2; lane 4, CMV-Cdk2; lane 5, CMV-Cdk3; lane 6, CMV-Cdk4; lane 7, CMV-Cdk5; lane 8, CMV-Cdk6; lane 9, CMV-PCTAIRE. NMS, normal mouse serum. **d**, Cdk5 can be activated by purified p35 expressed in bacteria but not by bacterially produced GST fusion proteins of human cyclin A or cyclin B. Lane 1, 6 $\times$ His-Cdk5; lane 2, 6 $\times$ His-p35; lane 3, 6 $\times$ His-Cdk5 + 6 $\times$ His-p35; lane 4, 6 $\times$ His-Cdk5 + GST-cyclin A; lane 5 6 $\times$ His-Cdk5 + GST-cyclin B. **METHODS.** **a–c**, CMV-p35 construct was made with a PCR fragment encompassing the entire open reading frame. This fragment was sequenced to prove that it did not contain any PCR errors. Transient transfection was performed as described¹⁶. Cell lysates were

collected 24–48 h after transfection. **d**, To add six histidine residues to Cdk5 (N-terminal) and p35 (C-terminal), *cdk5* and *p35* inserts were excised from the CMV-neo vector and cloned into pQE8 and pQE12 (Qiagen) respectively. Recombinant proteins were purified by chromatography over Ni-NTA-agarose (Qiagen). Full-length human cyclin A-GST¹⁷ and cyclin B-GST (gift of S. Shiff) fusion proteins were purified by glutathione-agarose chromatography. All *in vitro* kinase assays were performed as described using 1 μ g of each recombinant protein. All kinase reactions containing 6 $\times$ His-Cdk5 resulted in a 34K phosphorylated band comigrating with 6 $\times$ His-Cdk5 itself (**d**). Thus, low levels of phosphate could be incorporated into Cdk5 nonspecifically as activation of Cdk5 by p35 did not enhance the phosphorylation of the 34K band.

kinase. We observed that when Cdk5 immunoprecipitates were prepared from brain lysates and used for the Cdk5 *in vitro* kinase assay, a 25K protein was the major endogenous substrate (Fig. 2c) rather than the 35K protein seen in neuronal culture extracts. To test the relationship between the 25K and 35K proteins, Lew and colleagues kindly provided us with the amino-acid sequence obtained by microsequencing purified bovine p25¹⁰. We used these sequences to design primers for polymerase chain reaction (PCR) and isolated related complementary DNAs from a human fetal brain cDNA library. All the cDNA clones contained overlapping sequences and represented independent isolates. The two largest clones (4.0 kilobases (kb) and 4.1 kb) contained an open reading frame of 307 amino acid residues (Fig. 2a) with a predicted M_r ~34.5K. The product of this cDNA was therefore named p35. p35 does not share obvious homology with any existing protein in the GenBank database, although extremely limited similarity with the cyclin box region of various cyclins can be found¹⁰.

The predicted amino-acid sequence encoded by this human cDNA is closely related to the bovine protein characterized by Lew *et al.*¹⁰ with a discrepancy of only two amino acids. The sequences from the 25K protein are completely contained within the predicted protein sequences encoded by the p35 cDNAs (Fig. 2a and ref. 10) and it seems likely that the 25K peptide purified by Lew *et al.* is a proteolytic fragment¹⁰. During the preparation of this manuscript a partial protein sequence of a 23K subunit of tau kinase II was published¹⁸ which differs at only two positions from the 25K protein of Lew *et al.*

To determine whether p35 encodes the 35K Cdk5-associated protein, we raised a rabbit polyclonal antiserum against p35

and tested whether p35 and Cdk5 are associated *in vivo*. p35 immunoprecipitates were prepared from brain lysates and analysed on western blots with DC17, a monoclonal antibody to Cdk5. Cdk5 was detected in total brain extracts and in immunoprecipitations prepared with antibodies to p35 or Cdk5, but not when control antibodies were used (Fig. 2b).

Analysis of p35 immunoprecipitates isolated from either brain lysates or tissue culture cells transfected with the expression construct CMV-p35 revealed a potent associated kinase activity (Fig. 2c; see Fig. 3 for more details). This activity phosphorylated a 35K protein that comigrated with the 35K phosphoprotein present in Cdk5 immunoprecipitates prepared from both sources (Fig. 2c). This kinase activity increased in a fashion similar to the increasing levels of Cdk5 kinase activity during neural differentiation (data not shown). The partial proteolytic maps generated by *Staphylococcus aureus* V8 protease from the 35K protein associated with and phosphorylated by Cdk5 from a neuronal culture (data not shown), and the Cdk5 phosphorylated 35K protein from brain lysates (Fig. 2c), and Cdk5 phosphorylated p35 from CMV-p35 transfected cells (Fig. 2c), were all identical (Fig. 2d). In addition, the V8 partial proteolytic maps of phosphorylated p35 recognized by anti-p35 from both brain lysates and CMV-p35 transfected cells were identical to those of the Cdk5-phosphorylated 35K protein (Fig. 2e). These experiments show that the product of the p35 cDNA associates with Cdk5 *in vivo* and that p35 encodes the Cdk5-associated 35K protein found in brain extracts and primary neuronal cultures.

To test whether p35 regulates the activity of the Cdk5 kinase, p35 was introduced into C33A cervical carcinoma cells that express Cdk5 protein but lack detectable Cdk5 histone H1 kinase

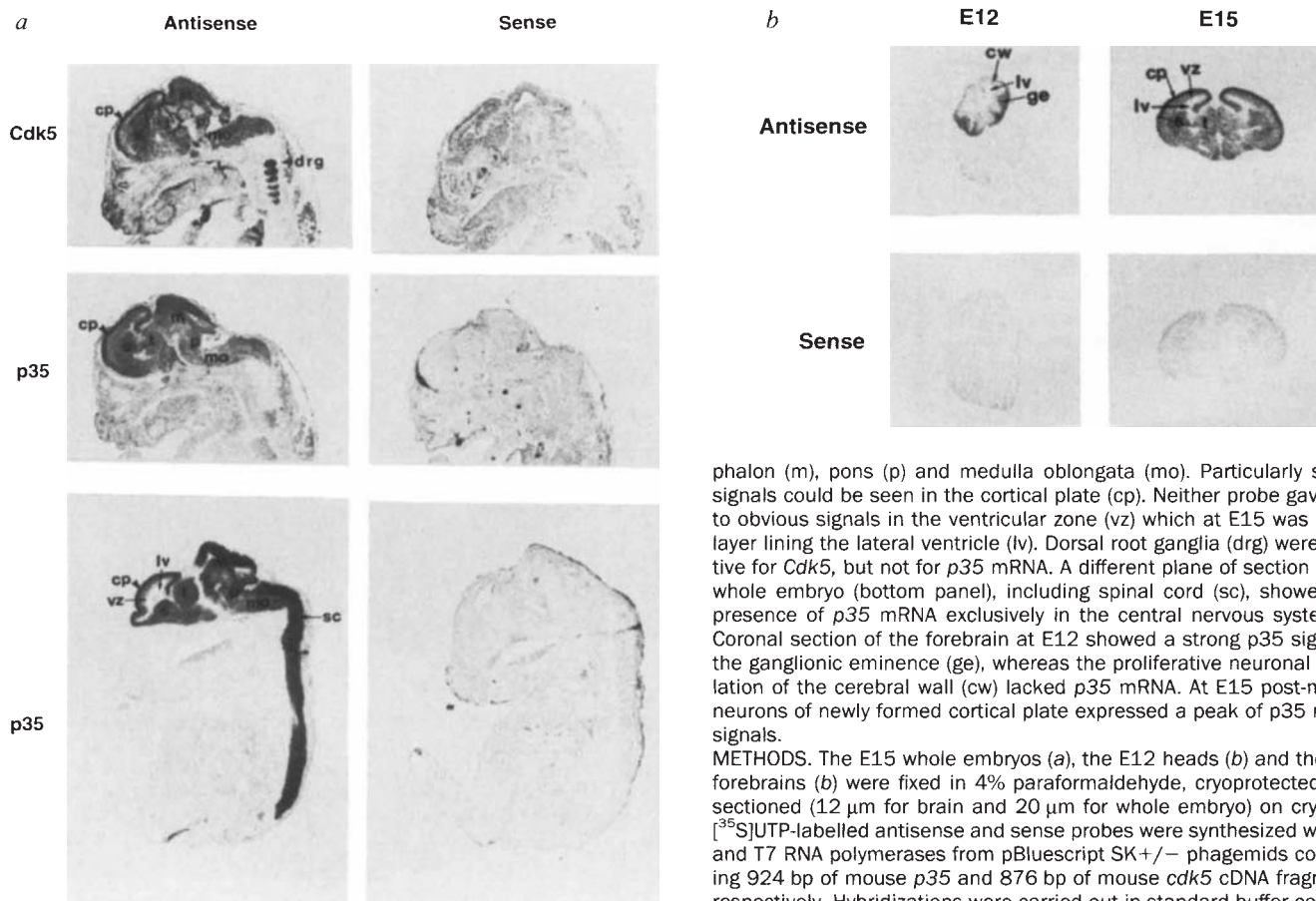


FIG. 4 Autoradiograms of *in situ* hybridization of embryonic mouse tissues with [³⁵S]UTP-labelled Cdk5 and p35 ribonucleotide antisense and sense probes. a, E15 mouse whole embryos. Both probes bound to parts of the central nervous system: striatum (s), thalamus (t), mesence-

phalon (m), pons (p) and medulla oblongata (mo). Particularly strong signals could be seen in the cortical plate (cp). Neither probe gave rise to obvious signals in the ventricular zone (vz) which at E15 was a thin layer lining the lateral ventricle (lv). Dorsal root ganglia (drg) were positive for Cdk5, but not for p35 mRNA. A different plane of section of the whole embryo (bottom panel), including spinal cord (sc), showed the presence of p35 mRNA exclusively in the central nervous system. b, Coronal section of the forebrain at E12 showed a strong p35 signal in the ganglionic eminence (ge), whereas the proliferative neuronal population of the cerebral wall (cw) lacked p35 mRNA. At E15 post-mitotic neurons of newly formed cortical plate expressed a peak of p35 mRNA signals.

METHODS. The E15 whole embryos (a), the E12 heads (b) and the E15 forebrains (b) were fixed in 4% paraformaldehyde, cryoprotected, and sectioned (12 μ m for brain and 20 μ m for whole embryo) on cryostat. [³⁵S]UTP-labelled antisense and sense probes were synthesized with T3 and T7 RNA polymerases from pBluescript SK+/- phagemids containing 924 bp of mouse p35 and 876 bp of mouse cdk5 cDNA fragments respectively. Hybridizations were carried out in standard buffer containing 50% formamide and 5×10^6 c.p.m. ml⁻¹ ³⁵S-labelled probes. Sections were rinsed in $2 \times$ SSC, incubated with RNase A (20 μ g ml⁻¹), and washed at 55 °C in progressively lower concentrations of SSC (2, 0.5 and 0.2 $\times$ SSC). The slides were dried and exposed to β -max film (Amersham) at 4 °C for 3 days. Magnifications: a, $\times 3.8$; b, $\times 4.4$.

activity⁷. Both PA1 (an antibody that recognizes the active Cdk5 kinase) and DC17 (antibody that does not recognize the active form of the kinase) were used to follow the Cdk5 complexes. When CMV-Cdk5 was introduced into the cells, both antibodies recognized the ectopically expressed Cdk5, but no noticeable histone H1 kinase activity was found in these immune complexes (Fig. 3a). When CMV-p35 was introduced into the cells, p35 was coprecipitated with Cdk5 by PA1 and these complexes exhibited histone H1 kinase activity. In contrast, DC17 did not recognize the active form of the kinase and failed to precipitate p35. Finally, cotransfection of CMV-Cdk5 and CMV-p35 expression vectors produced higher levels of association and histone H1 kinase activity. Thus p35 acts as an activator of the Cdk5 kinase.

To show that the kinase activity seen in the cotransfection assays was due to Cdk5 and not to a contaminating kinase, a dominant-negative mutant version¹² of Cdk5 (Asp 144 to Asn 144) was cotransfected with p35 (Fig. 3b). Similar forms of dominant-negative mutants of Cdk5 can block functional activity in transfection assays¹². Exogenously expressed Cdk5 protein was distinguished from the endogenous protein by means of an influenza haemagglutinin epitope (HA) tag (see lane 11 for example). As before, wild-type Cdk5 associated with p35 and produced an active kinase. The Cdk5 dominant-negative mutant also associated with p35, but completely abolished the associated kinase activity. These experiments strongly suggest that Cdk5 provides the active kinase present in the p35-Cdk5 complexes.

We next cotransfected various HA-tagged cyclin-dependent kinases¹² together with p35 (Fig. 3c). Although similar levels of the cyclin-dependent kinases were expressed, the coexpression of p35 only activated the histone H1 kinase activity of Cdk5. Thus, p35 seems to be a highly specific regulatory partner for Cdk5.

To test whether p35 alone is sufficient to activate Cdk5, recombinant Cdk5 and p35 were produced in bacteria, purified, and assayed for kinase activity. Although neither Cdk5 nor p35 preparations possessed kinase activity, a potent histone H1 kinase was generated when these two components were combined (Fig. 3d). Cdk5 was not activated by incubation with either glutathione-S-transferase (GST)-cyclin A or GST-cyclin B fusion proteins. This experiment shows that p35 can directly activate Cdk5 but does not exclude the involvement of other proteins in the activation process *in vivo*.

To study the temporal and spatial expression patterns of p35 and Cdk5 during embryonic development by *in situ* hybridization, E12 and E15 mouse whole embryos and coronal sections of the forebrain were hybridized with p35 and Cdk5 ribonucleotide probes. p35 expression, detected by the antisense p35 probe, was restricted to the central nervous system (CNS), including cerebral cortex, striatum, thalamus, midbrain, hindbrain and spinal cord in the E15 embryos. The control sense probe gave no specific signal (Fig. 4a, middle and bottom panels). Cdk5 expression pattern (Cdk5 antisense probe; Fig. 4a, top panel) closely resembled that of p35 but was not identical. In particular, the dorsal root ganglia showed significant Cdk5 expression but no p35 expression (Fig. 4a, top and middle panels). At E12, p35 antisense probe gave rise to obvious signals in the ganglionic eminence containing a mixture of post-mitotic neurons and proliferating cells and weak signals in the cerebral wall containing mostly proliferating cells (Fig. 4b). By E15, the highest level of p35 signal was observed in the cortical plate containing only post-mitotic neurons and again, the signals were much weaker in the ventricular zone which contains mostly proliferating cells (Fig. 4b). This distribution of p35 in the developing forebrain corresponds closely to that of Cdk5 (ref. 7; Fig. 4a) and suggests that both are most highly expressed in post-mitotic neurons. p35 seems to be expressed only in the CNS however, whereas Cdk5 is expressed in both the CNS and the peripheral nervous system^{7,11} (Fig. 4a).

The overlapping expression patterns of p35 and Cdk5, the association of p35 and Cdk5, and the observation that p35 is a

potent and specific activator of Cdk5 kinase, all show that p35 meets all the criteria for a regulatory subunit for Cdk5. The primary structure of p35, however, is only marginally similar to that of the known vertebrate cyclins and p35 is expressed at a highest level in the post-mitotic neurons rather than the proliferating cells. Therefore, p35 seems to be a novel type of regulatory partner for the Cdk5. In addition, the data provide the first instance of the biological activity of a vertebrate Cdk and its regulatory partner in terminally differentiated cells. □

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A brain-specific activator of cyclin-dependent kinase 5

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PHOSPHORYLATION of the neurofilament proteins of high and medium relative molecular mass, as well as of the Alzheimer's tau protein, is thought to be catalysed by a protein kinase with Cdc2-like substrate specificity^{1–7}. We have purified a novel Cdc2-like kinase from bovine brain⁸ capable of phosphorylating both the neurofilament proteins⁹ and tau¹⁰. The purified enzyme is a heterodimer of cyclin-dependent kinase 5 (Cdk5)⁹ and a novel regulatory subunit, p25 (ref. 8). When overexpressed and purified from *Escherichia coli*, p25 can activate Cdk5 *in vitro*. Unlike Cdk5, which is ubiquitously expressed in human tissue, the p25 transcript is expressed only in brain. A full-length complementary DNA clone showed that p25 is a truncated form of a larger protein precursor, p35, which seems to be the predominant form of the protein in crude brain extract. Cdk5/p35 is the first example of a Cdc2-like kinase with neuronal function.

The catalytic subunit of the neuronal Cdc2-like kinase has been isolated previously^{9,11–13}. Using the polymerase chain

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cyclin consensus	L Q L V G . . A M F L A S K Y E E
p25	L Q A V L L T C L V L S Y S V M G
CLN1	A K L V V G T C L W L A A K T W G
ORFD	R H R I F L G C L I L A A K T L N
HCS26	I H R I F E A C L I L S A L F H N
PHO80	A H R F L L T A T T V A T K G L C

FIG. 2 Comparison of p25 with cyclin and cyclin-like sequences. The cyclin consensus sequence is derived from cyclins A, B, D and E, with the highly conserved L and K residues highlighted with asterisks. Shaded residues indicate matches to p25.

2). When the Owl database was scanned with this sequence, no cyclin or cyclin-related protein appeared in the top 100 'hits'. Thus, on sequence comparison alone, it is far from clear that p25 is even a distant relative of the cyclin family.

As the neuronal Cdc2-like kinase is composed of no detectable subunits other than Cdk5 and p25, the p25 may carry out a cyclin-like function. To test for functional activity, GST-p25 and GST-Cdk5 fusion proteins were individually expressed in *E. coli*, purified by glutathione-agarose chromatography, reconstituted, and then analysed for kinase activity. Although neither of the bacterially expressed proteins alone showed any activity, reconstitution of GST-p25 together with GST-Cdk5 resulted in high kinase activity towards a synthetic peptide substrate, as well as increased autophosphorylation of p25 (Fig. 3). Although the specific enzyme activity was low, further purification of the reconstituted sample resulted in an enzyme preparation whose

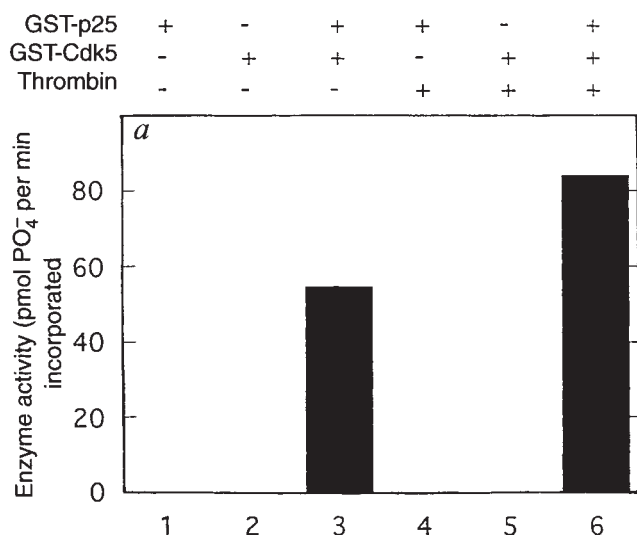


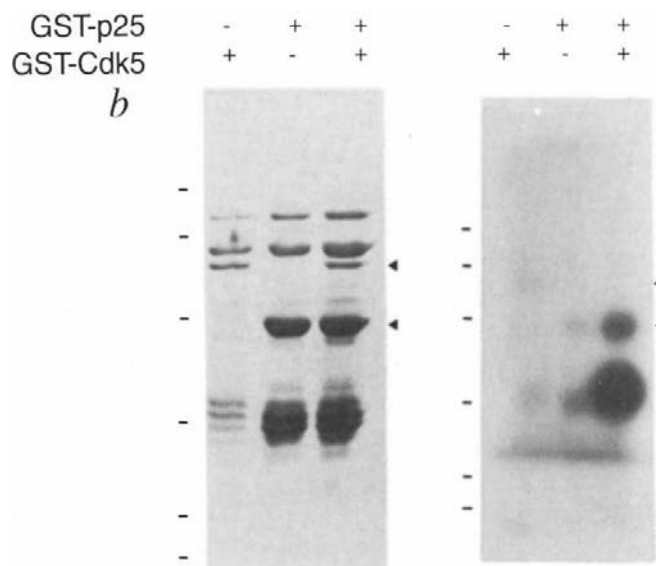
FIG. 3 Reconstitution of neuronal Cdc2-like kinase activity from purified components. Bacterially expressed GST-p25 and GST-Cdk5 fusion proteins were preincubated alone or together, and kinase activity assayed as described below. **a**, Histone peptide kinase activity before and after thrombin cleavage. **b**, Autophosphorylation of the p25 subunit. Left, SDS-PAGE analysis of expressed proteins. Gel is stained with Coomassie blue. Right, autoradiograph of ³²P-labelled proteins. Upper and lower arrow heads indicate GST-Cdk5 and GST-p25, respectively. Relative molecular mass markers from top to bottom are: 97.4K, 66.2K, 45K, 31K, 21.5K, 14.4K.

METHODS. *Bam*HI and *Eco*RI sites were engineered onto the 5' and 3' ends, respectively, of the longest 5'RACE fragment (T1-JL) by polymerase chain reaction using the following primers: 5'-cccggatcctgtcccccctgccagc-3'; 5'-cccgggaattctagttcttcaggtcggaga-3'. The resulting PCR product was subcloned into the expression plasmid, pGEX-2T, and this construct transformed into *E. coli* strain BL21 (DE3). This construct encodes a protein composed of GST fused to a fragment (residues 108-292) of p25 (GST-p25). A 500-ml culture was grown to an A₆₀₀ ~0.8 at 37 °C, when synthesis of GST-p25 was induced by 0.2 mM isopropyl-β-D-thiogalactoside at 25 °C for 16 h. Cells were collected and GST-p25 purified as described in ref. 23 with the following

specific activity was similar to that of neuronal Cdc2-like kinase purified from brain (5-10 μmol min⁻¹ mg⁻¹). When GST-Cdk5 was incubated with GST alone, the sample was totally devoid of kinase activity (not shown). This result suggests that, unlike the case with other cyclin-dependent kinases^{17,19}, full activation of Cdk5 occurs upon binding of p25 alone without the requirement for exogenous phosphorylation. It cannot rule out, however, the possibility that phosphorylation at Ser 159 (equivalent to Thr 161 in p34^{cdc2}) *in vivo* may result in further enzyme activation.

Northern analysis of bovine brain messenger RNA revealed single bands of around 1.3 kilobases (kb) and 4 kb, when probed with Cdk5 and p25 cDNA respectively (Fig. 4a, b). Cdk5 was found to be expressed relatively equally in all human tissues with the exception of brain, in which expression was several-fold greater (Fig. 4c). In contrast, p25 was expressed exclusively in brain, where transcripts of ~4 kb and ~2.4 kb were detected (Fig. 4d). Autoradiography for 10 times the exposure shown in Fig. 4d revealed no detectable p25 mRNA in tissues other than brain (not shown).

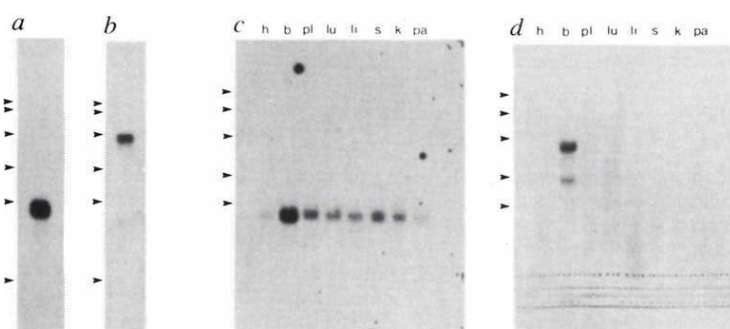
The large transcript encoding p25 raised the possibility that the purified protein was a proteolytic product of a larger precursor molecule. A cDNA clone of ~3.6 kb was therefore isolated from a bovine brain cDNA library and sequenced. This clone contained a single open reading frame encoding a protein of 307 amino acids (*M*_r 33.7K). The deduced sequence corresponded exactly to that of p25, plus an additional 98 amino



modifications. Cells were lysed using a French press (1,000 p.s.i.). After centrifugation, 10 ml supernatant were loaded onto 2 ml GSH-Sepharose (Pharmacia) by constant end-over-end rotation at 4 °C for 1 h. Full-length Cdk5 was expressed as a GST fusion protein in the expression plasmid pGEX-2TK (a gift of L.-H. Tsai). Expression and purification were performed as described for GST-p25. Reconstitution of expressed GST-p25 with expressed GST-cdk5 was carried out as follows: purified GST-Cdk5 (20 pmol) and GST-p25 (130 pmol) were preincubated either alone or together in 30 μl PBS (190 mM), dithiothreitol (1 mM), and EDTA (1 mM), pH 7.2, for 2 h at 22 °C in the presence or absence of thrombin (10 U per mg recombinant protein). Three microlitres of the preincubation mixture were diluted to 30 μl with phosphorylation reaction mixture (5 mM MgCl₂, 30 mM MOPS (pH 7.2), and 100 μM histone peptide), and assayed for kinase activity. Reactions were initiated by the addition of 100 μM [³²P]-ATP (350 d.p.m. pmol⁻¹), and continued for 20 min at 30 °C. The incorporation of ³²P into peptide was quantitated as previously described⁸. For p25 autophosphorylation, preincubation was for 1 h at 30 °C followed by phosphorylation with 100 μM [³²P]-ATP (6,800 d.p.m. per pmol) for 40 min. Proteins were resolved by SDS-PAGE and autoradiographed. The phosphorylated band at ~31K is bacterial protein.

FIG. 4 Northern blot analysis and tissue distribution of neuronal Cdc2-like kinase subunit mRNAs. mRNA from bovine brain (a, b) or from human tissues (c, d) was resolved by agarose-gel electrophoresis and probed with a radiolabelled cDNA corresponding to either Cdk5 (a, c) or p25 (b, d). *M_r* markers are 9.5, 7.5, 4.4, 2.4, 1.4 kb (and 0.24 kb for bovine blots). h, Heart; b, brain; pl, placenta; lu, lung; li, liver; s, skeletal muscle; k, kidney; pa, pancreas.

METHODS. a, b, Total bovine brain RNA was prepared²⁵ and the poly(A)⁺ fraction was isolated using the Poly(A)⁺ Tract System from Promega. Two micrograms poly(A)⁺ RNA were resolved by gel electrophoresis (1.2% agarose) and blotted to Hybond N+ membrane (Amersham) by capillary action. Blots were baked for 2 h at 80 °C and hybridized with radiolabelled cDNA probe in 5× SSPE (750 mM NaCl, 50 mM NaH₂PO₄, 5 mM EDTA, pH 7.4), 5× Denhart's, 0.1% SDS for 16 h at 68 °C, after prehybridization under the same conditions for 1 h. Blots were washed at 68 °C in 2× SSPE for 10 min, twice in 1× SSPE for 10 min, twice in 0.1× SSPE for 10 min, and then autoradiographed at -70 °C. Exposure times were 24 h (a), 4 days (b). c, d, A northern blot of human tissues was purchased from Clontech.



Hybridization was carried out as for bovine blots except at 60 °C. Blots were washed at 60 °C in 2× SSPE for 10 min, twice in 1× SSPE for 15 min, and then autoradiographed at -70 °C. Exposure times were 20 h (c), and 36 h (d). cDNA probes were 5'-RACE fragments corresponding to either T2D⁹ (a, c) or T1-JL (b, d).

acids at the N terminus (Fig. 1). Using antisera raised against recombinant p25, we analysed crude and partially purified brain extracts by western blotting for the presence of a putative p25 precursor protein. In the 120,000g supernatant fraction of bovine brain, anti-p25 antibodies strongly stained a protein band of ~35K, whereas no staining of a band at 25K was detected. Immunoreactivity correlated with kinase activity and persisted as a 35K band in early steps during routine purification⁸, but shifted to a band of 25K after Superose-12 chromatography (data not shown). These results suggest that p25 is the proteolytic product of a precursor protein, p35. Tsai *et al.*²⁰ have shown that p35 is the predominant form of this protein in cultured neuronal extracts, and that immunoprecipitates of the Cdk5-p35 complex show histone H1 kinase activity.

Purified neuronal Cdc2-like kinase phosphorylates both the neurofilament protein NF-M and NF-H at sites identical to those phosphorylated by p34^{cdc2} (ref. 9), and phosphorylates tau exclusively at sites phosphorylated in Alzheimer's disease¹⁰. Whereas neither of the kinases p34^{cdc2} nor p33^{cdk2} are present in brain^{11,21}, both Cdk5 and p25/p35 are expressed predominantly in the central nervous system^{11,13,20}. Furthermore, Cdk5 colocalizes with neurofilament tracts in the axons of neuronal cells in culture²². This makes the neuronal Cdc2-like kinase a strong candidate for catalysing neurofilament and tau phosphorylation *in vivo*. The neuronal Cdc2-like kinase is the first example of a Cdc2-like kinase activity in neurons, and supports the idea that members of the Cdc2 family are involved in processes other than cell-cycle control. □

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Activation of p70/p85 S6 kinase by a pathway independent of p21^{ras}

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THE enzymes p70^{s6k} and p85^{s6k} are two isoforms of the same kinase^{1,2} and are important in mitogenesis²⁻⁴. Both isoforms are activated by a complex phosphorylation event⁵ and lie on a common signalling pathway⁴, distinct from that of the p42^{mapk}/p44^{mapk} kinases⁶. Activation of p42^{mapk}/p44^{mapk} is triggered by sequential activation of the GDP-GTP exchange factor Sos, the GTP-binding protein p21^{ras}, and protein kinases p74^{raf} and p47^{mek} (refs 7-10). As p21^{ras} transformed cells have increased S6 phosphorylation¹¹, we tested whether the p70^{s6k}/p85^{s6k} signalling pathway bifurcates between p21^{ras} and p42^{mapk}/p44^{mapk}. We found that mutants of p74^{raf} and p21^{ras} blocked activation of epitope-tagged p44^{mapk} but not epitope-tagged p70^{s6k}. Moreover, in cells expressing human platelet-derived growth factor receptors lacking

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the kinase-insert domain, the growth factor activates $p21^{ras}$ but not $p70^{s6k}/p85^{s6k}$. The critical autophosphorylation site for $p70^{s6k}/p85^{s6k}$ activation within this domain is a tyrosine at residue 751. Our results show that the $p70^{s6k}/p85^{s6k}$ signalling pathway is independent of $p21^{ras}$, that it bifurcates from the $p21^{ras}$ pathway at the receptor, and that it is initiated by autophosphorylation at a specific site.

A number of mitogens were initially tested for their ability to activate $p42^{mapk}/p44^{mapk}$ and $p70^{s6k}/p85^{s6k}$ in 293 cells, the vehicle used for transient transfection. Serum, epidermal growth factor (EGF) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) all rapidly activated both kinase families from low basal levels (Fig. 1a and c, lanes 1–3 and 6), consistent with their reduced mobility on SDS-PAGE (Fig. 1b and d, lanes 1–3 and 6). Platelet-derived growth factor (PDGF) had no effect on either enzyme (Fig. 1a–d, lane 4), whereas insulin was found to stimulate $p70^{s6k}/p85^{s6k}$ (Fig. 1a and b, lane 5) but not $p44^{mapk}$ (Fig. 1c and d, lane 5) or $p42^{mapk}$ (data not shown). Thus $p70^{s6k}/p85^{s6k}$ activation can be dissociated from $p42^{mapk}/p44^{mapk}$ activation, in agreement with earlier findings^{4,6}, and EGF is the only growth factor capable of activating both enzymes.

As $p74^{raf}$ is the most proximal serine/threonine kinase to the EGF receptor¹² and therefore the most likely point of bifurcation, we co-expressed the regulatory portion of $p74^{raf}$, amino acids 1–259 ($N\Delta raf$)¹³, in 293 cells with either an influenza

haemagglutinin (HA) protein epitope-tagged $p44^{mapk}$ or Myc-tagged $p70^{s6k}$ (ref. 14). Both EGF and TPA induce $p44^{mapk}$ -HA and $p70^{s6k}$ -Myc activation in the presence of the vector alone (Fig. 2a and c; compare lane 1 with 2 and 3), an effect mirrored by decreased electrophoretic mobility (Fig. 2b and d). In the presence of $N\Delta raf$, activation of $p44^{mapk}$ -HA by EGF or TPA is prevented (Fig. 2a and b, lanes 5 and 6), but not the activation of $p70^{s6k}$ -Myc (Fig. 2c and d, lanes 5 and 6), as measured by kinase assay or mobility shift. The results argue that $p74^{raf}$ is not a component of either the EGF- or TPA-signalling pathway leading to $p70^{s6k}/p85^{s6k}$ activation, suggesting that bifurcation of the two pathways is upstream of $p74^{raf}$.

Because $p21^{ras}$ can signal to $p42^{mapk}/p44^{mapk}$ independently of $p74^{raf}$ (ref. 15), $p21^{ras}$ may signal to $p70^{s6k}/p85^{s6k}$ independently of $p74^{raf}$, consistent with increased levels of S6 phosphorylation in H-ras-transformed cells¹¹. To assess the role of $p21^{ras}$ in $p70^{s6k}/p85^{s6k}$ activation, we used N17ras, an interfering mutant of $p21^{ras}$ (ref. 16). Coexpression of N17ras with $p44^{mapk}$ -HA in 293 cells blocks EGF-induced activation of the kinase (Fig. 3A, a, compare lanes 2 and 5) and its mobility shift on SDS-PAGE (data not shown). Figure 3A (a, lanes 3 and 6) shows that the inhibition by N17ras can be circumvented by TPA in 293 cells, presumably as a result of direct activation of $p74^{raf}$ by protein kinase C¹⁷, indicating that the pathway is intact from $p74^{raf}$ downstream. Unexpectedly, no effect of N17ras is observed on EGF- or TPA-induced $p70^{s6k}$ -Myc activation (Fig. 3A, b), indicating that the point of bifurcation is more proximal to the receptor. This result, however, does not exclude a requirement for $p21^{ras}$ in $p70^{s6k}/p85^{s6k}$ activation by other mitogens. We therefore tested NIH3T3 cells (A14) or porcine aortic endothelial cells (PAE) expressing either the insulin¹⁸ or PDGF β -type receptors¹⁹, respectively, for ligand-induced $p42^{mapk}/p44^{mapk}$ and $p70^{s6k}/p85^{s6k}$ activation. In the presence of N17ras, both insulin- (Fig. 3B, a) and PDGF- (Fig. 3B, c) induced $p44^{mapk}$ -HA activa-

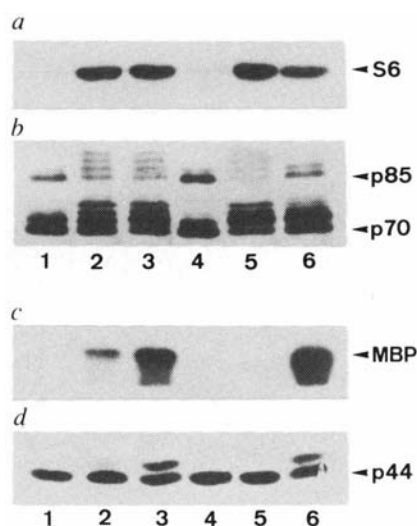


FIG. 1 Activation of $p70^{s6k}/p85^{s6k}$ and $p44^{mapk}$ following mitogenic stimulation of 293 cells. S6 (a) and myelin basic protein (MBP) kinase (c) activities were measured following immunoprecipitation with specific antibodies against either $p70^{s6k}/p85^{s6k}$ or $p44^{mapk}$, respectively. Mobility shift of $p70^{s6k}/p85^{s6k}$ (b) or $p44^{mapk}$ (d) was detected on western blots with the corresponding antibody. Cell lysates were prepared from either untreated cells (lane 1), or cells treated for 10 min with 10% FCS (lane 2), 5 nM EGF (lane 3), 1 nM PDGF (lane 4), 1 μ M insulin (lane 5) or 5 μ M TPA (lane 6).

METHODS. Human 293 cells were seeded at 10^6 cells per 10-cm dish in DMEM containing 10% FCS. After 36 h, cells were further cultured for 24 h in DMEM containing 0.5% FCS followed by an additional 8 h in serum free DMEM. Cultures were then stimulated with specific mitogens, lysed and extracts prepared⁵. For S6 kinase assays, 20 μ g cell lysate were immunoprecipitated with the $p70^{s6k}/p85^{s6k}$ M5 antibody and assayed according to ref. 27. For MBP kinase assays, 200 μ g cell lysate was immunoprecipitated with 10 μ l of a specific $p44^{mapk}$ polyclonal antibody (F15P antibody; D. Fabbro, unpublished) directed against the last 15 amino acids of the kinase²⁸. Immunoprecipitates were then assayed for MBP activity according to ref. 18, except that 15 μ g instead of 7.5 μ g MBP was used as substrate. Western blot analysis of $p70^{s6k}/p85^{s6k}$ and $p44^{mapk}$ was carried out as described³, except in the case of $p44^{mapk}$ for which 10 μ g total cell lysate was analysed using F15P antibody at 1:2,000 dilution.

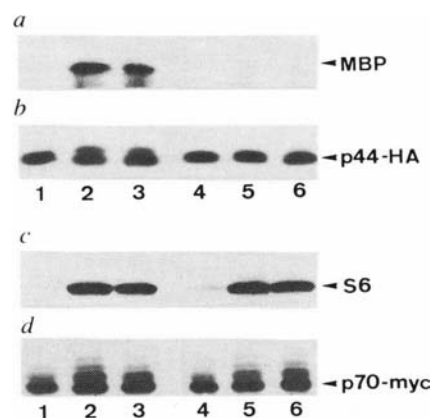


FIG. 2 $N\Delta raf$ does not inhibit EGF- and TPA-induced $p70^{s6k}$ activation. $p44^{mapk}$ -HA (a) or $p70^{s6k}$ -Myc (c) activity and either $p44^{mapk}$ -HA (b) or $p70^{s6k}$ -Myc (d) mobility shifts were analysed from extracts of untreated cells (lanes 1 and 4) or cells stimulated with either 5 nM EGF (lanes 2 and 5) or 5 μ M TPA (lanes 3 and 6). Before stimulation, cultures were co-transfected with 1 μ g each of the $p70^{s6k}$ -Myc and $p44^{mapk}$ -HA plasmids in the presence of 18 μ g of either the vector plasmid (lanes 1–3) or the vector plasmid containing $N\Delta raf$ (lanes 4–6).

METHODS. Cells were cultured as for Fig. 1. Plasmid DNA was added 24 h after seeding and removed 12 h later⁵, when cells were cultured in DMEM containing 0.5% serum. Kinase was assayed as for Fig. 1, except that 12CA5 and 9E10 antibodies¹⁴ were used to immunoprecipitate $p44^{mapk}$ -HA and $p70^{s6k}$ -Myc. For western blots, $p44^{mapk}$ -HA and $p70^{s6k}$ -Myc were detected with either F15P or M1 antibody (Fig. 1 legend). The Myc epitope tag¹⁴ was inserted at the N terminus of $p70^{s6k}$ and subcloned into a human cytomegalovirus-promoter-driven expression vector⁵. The $p44^{mapk}$ -HA vector construct has been described²⁹.

tion is blocked, with no corresponding effect on $p70^{s6k}$ -Myc activation (Fig. 3*B*, *b* and *d*), consistent with the hypothesis that tyrosine kinase receptors do not use $p21^{ras}$ in $p70^{s6k}$ / $p85^{s6k}$ activation.

To investigate whether a $p21^{ras}$ requirement in $p70^{s6k}$ / $p85^{s6k}$ activation could be masked by redundant signalling pathways, we used NMuMG cells expressing either the human PDGF receptor or the same receptor lacking the kinase insert domain (ΔKi)²⁰. Treatment of either cell type with PDGF led to a 2–3-fold activation of $p21^{ras}$ (Fig. 4*A* and ref. 21), and a similar activation of $p42^{mapk}$ / $p44^{mapk}$ (Fig. 4*B*, *a* and ref. 21), whereas the ΔKi mutant failed to activate $p70^{s6k}$ / $p85^{s6k}$ (Fig. 4*B*, *b*). Thus $p21^{ras}$ activation is not sufficient for activation of $p70^{s6k}$ / $p85^{s6k}$.

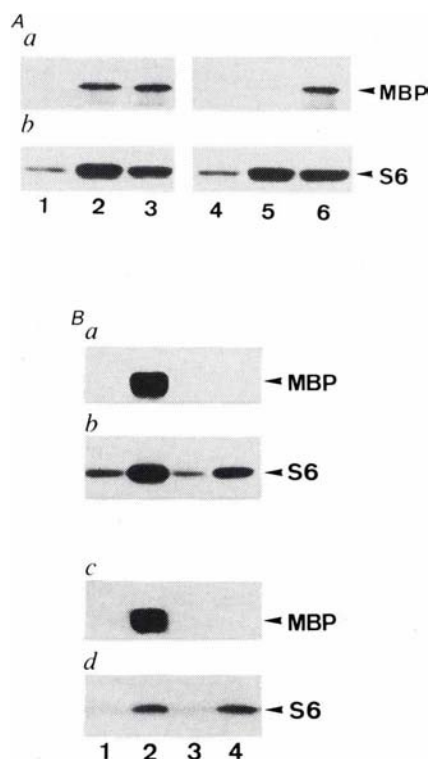


FIG. 3 Mitogen-induced activation of $p70^{s6k}$ is not inhibited by N17*ras*. **A**, 293 cell extracts were analysed for either $p44^{mapk}$ -HA (*a*) or $p70^{s6k}$ -Myc (*b*) activation from untreated cells (lanes 1 and 4), or cells stimulated for 10 min with 5 nM EGF (lanes 2 and 5) or 5 μ M TPA (lanes 3 and 6). Before stimulation, cultures were co-transfected with 5 μ g each of the $p70^{s6k}$ -Myc and $p44^{mapk}$ -HA plasmid in the presence of 10 μ g of either the vector plasmid (lanes 1–3) or the vector plasmid containing N17*ras* (lanes 4–6). **B**, A14 (*a* and *b*) and PAE cells, the latter expressing PDGF β -receptors, (c and d) were co-transfected with $p70^{s6k}$ -Myc ($p70^{s6k}$ -HA in the case of A14 cells) and $p44^{mapk}$ -HA in the presence of the vector plasmid (lanes 1 and 2) or the vector plasmid containing N17*ras* (lanes 3 and 4). Lysates were prepared from untreated cells (*a*–*d*, lanes 1 and 3) or cells stimulated for 10 min with either 1 μ M insulin (*a* and *b*, lanes 2 and 4) or 1 nM PDGF (*c* and *d*, lanes 2 and 4).

METHODS. 293 cells were cultured and transfected as for Fig. 2. A14 or PAE cells were grown and maintained as described^{18,19}. For transfection of A14 cells, 2 μ g each plasmid construct was used. After transfection and before insulin stimulation, cells were cultured for 24 h in DMEM containing 2% FCS. For transfection of PAE cells expressing PDGF β -receptors, 5 μ g of each kinase plasmid vector and 10 μ g of vector or vector containing N17*ras* were used. Transfection of PAE cells expressing PDGF β -receptors was done as for 293 cells except that after transfection cells were cultured in DMEM for 24 h without FCS. N17*ras* was subcloned into an RSV-LTR promoter-driven expression vector²¹.

To determine if an autophosphorylation site in the ΔKi domain might be essential for initiating the $p70^{s6k}$ / $p85^{s6k}$ pathway, we tested for kinase activation in PAE cells stably transfected with PDGF receptors containing point mutations at sites known to bind specific signalling molecules, including Y716 (growth factor receptor binding protein 2, GRB2)²², Y740/Y751 (phosphatidylinositol-3-OH kinase, PI(3)K)^{20,23}, Y751 (NCK)²⁴ and Y771 (GTPase-activating proteins, GAP)^{20,23}. The results show that mutations at Y740/Y751 or Y751, but not Y716, Y740 or Y771, significantly block $p70^{s6k}$ / $p85^{s6k}$ activation (Fig. 4*C*). Furthermore, the Y740 mutant has little effect on $p70^{s6k}$ / $p85^{s6k}$ activation, despite the fact that it suppresses receptor PI(3)K association to the same extent as the Y751 mutant (Fig. 4*D*),

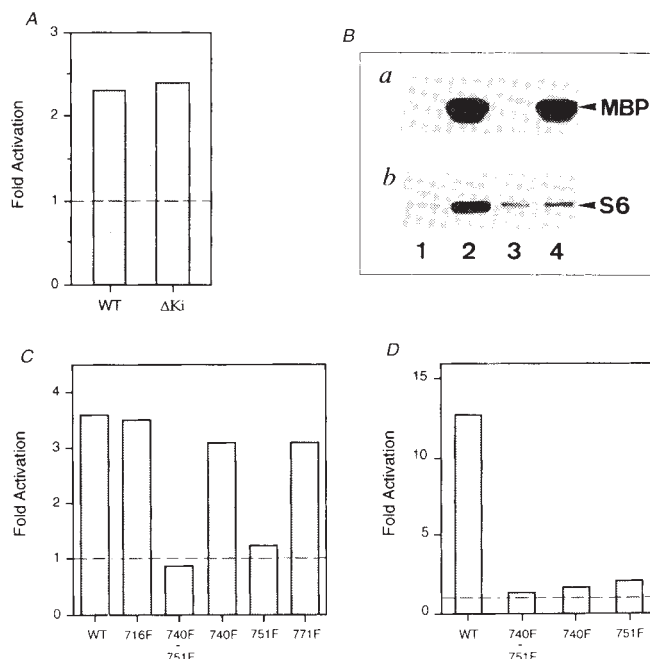


FIG. 4 PDGF activation of $p21^{ras}$, $p42^{mapk}$ / $p44^{mapk}$, $p70^{s6k}$ / $p85^{s6k}$ and PI(3)K in cells expressing PDGF-receptor mutants. **A**, Relative activation of $p21^{ras}$ by PDGF was measured in permeabilized NMuMG cells expressing either wild-type (WT) PDGF receptor or the same receptor lacking the kinase insert domain (ΔKi). **B**, *a*, $p42^{mapk}$ / $p44^{mapk}$ or *b*, $p70^{s6k}$ / $p85^{s6k}$ activity in extracts of NMuMG cells expressing either WT PDGF receptor (lanes 1 and 2) or the ΔKi mutant (lanes 3 and 4) was measured in the absence (lanes 1 and 3) or presence of PDGF (lanes 2 and 4). **C**, Relative activation of $p70^{s6k}$ / $p85^{s6k}$ by PDGF was measured in PAE cells expressing either the (WT) PDGF receptor or the same receptor expressing the indicated point mutations in which tyrosine was replaced by phenylalanine (F). **D**, Relative activation by PDGF of PDGF-receptor-associated PI(3)K activity was measured in PAE cells expressing either the WT PDGF receptor or the same receptor with the indicated point mutations in which tyrosine was replaced by phenylalanine. Lysates were prepared from cells treated with 1 nM PDGF- $\beta\beta$. **METHODS.** PAE or NMuMG cells were grown and maintained as described^{16,24}. $p21^{ras}$ guanine-nucleotide-exchange activity was measured in permeabilized NMuMG cells as described¹⁶. In brief, cells that had been stimulated with or without PDGF for 5 min at 4 °C were permeabilized with 0.1% digitonin in the presence of 10 μ M [α -³²P]GTP and incubated for 5 min at 37 °C. Cells were lysed, $p21^{ras}$ immunoprecipitated with monoclonal antibody Y13-259, and bound nucleotide measured. The labelled guanine nucleotide value for unstimulated cells was between 150–250 c.p.m. $p70^{s6k}$ / $p85^{s6k}$ were assayed as for Fig. 1. NMuMG cells were stimulated for 10 min and PAE cells for 30 min. The value for $p70^{s6k}$ / $p85^{s6k}$ activity in unstimulated PAE cells was between 0.45–0.67 pmol min⁻¹ mg⁻¹ protein. Receptor-associated PI(3)K activity was measured as described³⁰, the value for unstimulated cells being 31.4×10^3 – 37.9×10^3 arbitrary PhosphorImager units.

suggesting that another molecule is triggering this pathway, such as NCK.

Our results show that $p70^{s6k}/p85^{s6k}$ activation is independent of $p21^{ras}$ and that bifurcation of these two pathways occurs at the level of the receptor. The critical residue in initiating $p70^{s6k}/p85^{s6k}$ activation in the PDGF receptor in PAE cells is Y751, consistent with earlier findings²⁵. These results, however, do not support involvement of $p85/p110$ PI(3)K recruitment to the receptor nor the PI(3)K activity operating downstream of $p21^{ras}$ (ref. 26) in $p70^{s6k}/p85^{s6k}$ activation. The task will be to identify the putative $p70^{s6k}/p85^{s6k}$ signalling molecule that docks at Y751. □

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Interaction between two homeodomain proteins is specified by a short C-terminal tail

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Two yeast homeodomain proteins, $\alpha 1$ and $\alpha 2$, interact and cooperatively bind the haploid-specific gene (*hsg*) operator, resulting in the repression of a set of genes involved in the determination of cell type^{1–5}. The cooperative binding of $\alpha 1$ and $\alpha 2$ to DNA can be reconstituted *in vitro* using purified fragments of $\alpha 1$ and $\alpha 2$. Only the homeodomain is needed for $\alpha 1$, but for $\alpha 2$ a C-terminal 22-amino-acid tail is required as well^{4,6–9}. As most of the specificity of DNA binding appears to derive from $\alpha 1$, we proposed⁴ that $\alpha 2$ functions in the $\alpha 1/\alpha 2$ heterodimer to contact $\alpha 1$ with its tail. By construction and analysis of several chimaeric proteins, we investigate how two DNA-binding proteins, one with low intrinsic specificity ($\alpha 2$) and one with no apparent intrinsic DNA-binding ability ($\alpha 1$), can together create a highly specific DNA-binding activity⁴. We show that the 22-amino-acid region of $\alpha 2$ immediately C-terminal to the homeodomain, when grafted onto the $\alpha 1$ homeodomain, converts $\alpha 1$ to a strong DNA-binding protein. This $\alpha 2$ tail can also be attached to the *Drosophila engrailed* homeodomain, and the chimaeric protein now binds cooperatively to DNA with $\alpha 1$, showing how a simple change can create a new homeodomain combination that specifically recognizes a new DNA operator.

To test whether fusion of the $\alpha 2$ C-terminal 22-amino-acid tail onto the $\alpha 1$ homeodomain ($\alpha 1::\alpha 2$) can convert the $\alpha 1$ homeodomain into a form that binds DNA tightly and specifically in the absence of $\alpha 2$, we investigated the binding of the $\alpha 1::\alpha 2$ chimaeric protein to a synthetic $\alpha 1/\alpha 1$ operator (Figs 1 and 2a) and to an *hsg* operator (not shown). We found that the chimaera bound both operators efficiently. On the basis of the migration of the $\alpha 1::\alpha 2$ /DNA complex in gel mobility shift experiments⁴ and DNase I footprinting results (Fig. 2b), we conclude that the $\alpha 1::\alpha 2$ chimaera binds to DNA as a homodimer.

The simplest explanation of these results is that in each homodimer one molecule behaves as if it were $\alpha 1$ without an $\alpha 2$ tail attached, and the other molecule interacts with the first in the same way that $\alpha 2$ normally interacts with $\alpha 1$. To rule out the possibility that attachment of this tail to the end of the $\alpha 1$ homeodomain nonspecifically stabilizes the folding of the homeodomain and thereby allows $\alpha 1$ to bind to DNA, we constructed a similar chimaeric protein comprising the $\alpha 1$ homeodomain and an $\alpha 2$ tail that contains a single amino-acid substitution (leucine to serine at residue 196) known to abolish $\alpha 1/\alpha 2$ repression *in vivo*⁷. In contrast to the wild-type tail construct, this mutant construct bound only very weakly to the DNA (Fig. 2a), indicating that the interaction between the $\alpha 2$ tail and the $\alpha 1$ homeodomain is dependent on the amino-acid constitution of the tail, and that the results obtained with the chimaeric protein mirror the situation *in vivo*.

These results suggest that a novel combination of homeodomains could be constructed simply by grafting the tail of $\alpha 2$ onto a new homeodomain which should now bind DNA cooperatively with $\alpha 1$. To test this idea, the tail of $\alpha 2$ was grafted onto the *Drosophila engrailed* (*en*) homeodomain and the chimaera ($En::\alpha 2$) was tested for its ability to bind DNA cooperatively with the $\alpha 1$ homeodomain (Fig. 3). Under our conditions neither protein alone efficiently occupied a synthetic operator containing one binding site for $\alpha 1$ and one site for *engrailed* product (*En*). However, $En::\alpha 2$ and $\alpha 1$ together show strong cooperative binding, again suggesting that contacts between the tail of $\alpha 2$ and the homeodomain of $\alpha 1$ are all that are necessary for cooperative interaction. To rule out the possibility that the *En* homeodomain was responsible for the interaction with $\alpha 1$, we also tested for cooperative binding between $\alpha 1$ and the *En* homeodomain (not shown), and between $\alpha 1$ and the *En* homeodomain with its 40-amino-acid tail (Fig. 3). At high concentrations, both of these *En* proteins occupied the $\alpha 1/en$ operator by themselves, indicating that the proteins are active, but neither of them showed any cooperative binding with $\alpha 1$. From these results we conclude that the 22-amino-acid tail of $\alpha 2$ constitutes a domain that is sufficient for cooperative interaction with $\alpha 1$. This domain is functional when attached to $\alpha 1$ itself or to a heterologous homeodomain, conferring on the chimaeric protein the ability to interact with $\alpha 1$.

These experiments suggest that a large component of the DNA-binding specificity is contributed by the $\alpha 1$ protein, which, under our set of biochemical conditions, does not bind DNA on its own. For example, the complex composed of $\alpha 1$ and $En::\alpha 2$, in addition to binding the $\alpha 1/en$ operator, also recognizes the

hsg operator, but with reduced affinity (not shown). Similarly, the $\alpha 1/\alpha 2$ complex can efficiently bind to the *a1/en* operator (not shown) and to the *a1/a1* operator¹⁰, despite the fact that the preferred binding site for the En or $\alpha 2$ homeodomains is not present in these operators. Binding is improved when the correct recognition site for the homeodomain is present, indicating that some En and $\alpha 2$ homeodomain/DNA contacts do contribute to DNA-binding affinity.

It remains unclear how interaction with the $\alpha 2$ tail induces DNA-binding activity in $\alpha 1$, but recent NMR data⁹ show that the $\alpha 2$ tail undergoes a conformational change upon interaction with $\alpha 1$. The C-terminal tail of $\alpha 2$ is unstructured when $\alpha 2$ is in solution or when $\alpha 2$ is bound alone to the DNA^{11,12}. But when $\alpha 1$ and $\alpha 2$ come into contact, the tail of $\alpha 2$ becomes structured, forming an α -helix⁹. It is plausible that $\alpha 1$ undergoes a reciprocal conformational change upon interaction with the $\alpha 2$ tail. This change would take place in the $\alpha 1$ homeodomain, which contains the receptor for the $\alpha 2$ tail as well as the DNA-binding domain, and would convert $\alpha 1$ to a form competent to bind DNA. The amino acids in the turn between helices two and three of the $\alpha 1$ homeodomain may be potential targets for $\alpha 2$ interaction. Mutations in two of these amino acids (108 and 110) lower the cooperative binding of $\alpha 1/\alpha 2$ by 10–100-fold, whereas mutations in residues 80 and 87 of helix one and residues 98, 101 and 105

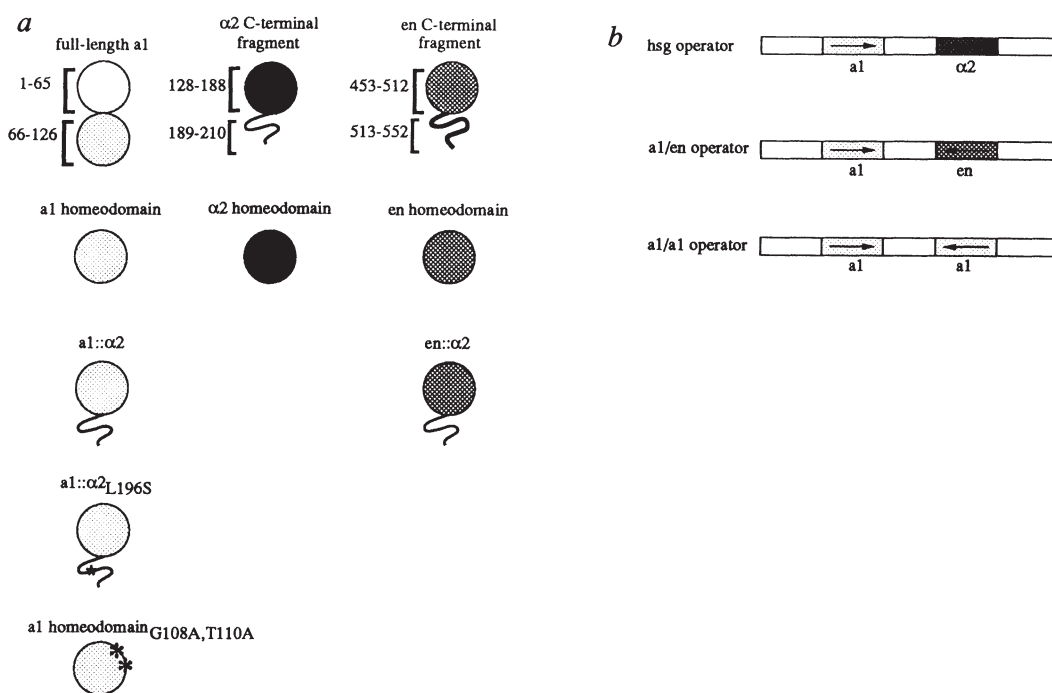
of helix two have no observable effect on the cooperative binding of $\alpha 1$ and $\alpha 2$ to DNA (Fig. 4). All of these residues are predicted, on the basis of the X-ray structure of $\alpha 2$ complexed to DNA¹², to be solvent-exposed and away from the DNA-binding surface, although we cannot rule out the possibility that a change in residues 108 or 110 produces a structural change in $\alpha 1$ that diminishes its interaction with DNA.

In addition to its interaction with $\alpha 1$, $\alpha 2$ binds DNA cooperatively with a second DNA-binding protein, MCM1 (ref. 13). The $\alpha 2$ /MCM1 interaction is similar in several respects to that between $\alpha 2$ and $\alpha 1$. In both, a short flexible region of $\alpha 2$ (for MCM1 this region is located immediately N-terminal to the $\alpha 2$ homeodomain¹⁴) interacts with the DNA-binding domain of MCM1 or $\alpha 1$. The C-terminal tail is not required for the $\alpha 2$ /MCM1 interaction⁶; likewise, the flexible region N-terminal to the $\alpha 2$ homeodomain is not required for interaction with $\alpha 1$ ⁴. Thus we can think of the $\alpha 2$ homeodomain as having two flexible extenders, one specifying interaction with $\alpha 1$, the other specifying interaction with MCM1 (Fig. 1c).

The cooperative interaction of UNC-86 and MEC-3, two *C. elegans* homeodomain proteins, bears many similarities to the $\alpha 1/\alpha 2$ interaction¹⁵. The POU homeodomain of UNC-86 is sufficient for both DNA binding and heterodimerization, whereas for MEC-3 a region containing the homeodomain plus

FIG. 1 Proteins (a) and operators (b) used in experiments. *hsg* ($\alpha 1/\alpha 2$): TCGACTATGATGACTTTTCTACATTGGGAAGC; *a1/en*: TCGACGACTATGATGACTTTTAAATTACCGAAGC; *a1/a1*: TCGATGATGAATTAAATCATCA. Binding sites are underlined and arranged with dyad symmetry. The recognition sequences for the three proteins are: $\alpha 1$, TGATGTA; $\alpha 2$, ATGTA; *en*, TAATT. The *hsg* operator is that from upstream of the *MATa1* gene⁴. The sequence of the *en* binding site was that used for the crystallization of the En homeodomain on DNA¹⁸.

c. Functional modules of $\alpha 2$. The short flexible region immediately N-terminal to the homeodomain (the hinge, residues 110–128) interacts with the core of MCM1¹⁴, the homeodomain binds to the DNA operator¹⁹, and the C-terminal tail interacts with the $\alpha 1$ homeodomain. In the absence of MCM1 and $\alpha 1$ the hinge and tail regions of $\alpha 2$ are unstructured^{6,11,12,20}. It has been shown that upon interaction with $\alpha 1$ the tail of $\alpha 2$ assumes an α -helical structure⁹. Although we have no experimental evidence, we assume that upon interaction with MCM1 the hinge of $\alpha 2$ also becomes structured. METHODS. All truncated and chimaeric versions of the proteins were generated by PCR mutagenesis²¹, using the full-length genes as templates. An *NdeI* site was introduced at the desired N terminus, providing the ATG necessary for translation. The $\alpha 1::\alpha 2$ chimaeras were made by ligating the homeodomain and $\alpha 2$ tail products from two separate PCR reactions, using a naturally occurring *BglII* site at the C terminus of $\alpha 1$. The *En::\alpha 2* chimaera was generated as follows. Two PCR products encoding the *en* homeodomain and the $\alpha 2$ tail were made to partially overlap in sequence at the desired junction between homeodomain and tail. These overlapping, primary products were denatured and allowed to reanneal, producing a heteroduplex product which was extended by *Taq* DNA polymerase to produce a fragment that is the sum of the two



overlapping products. This fragment was then amplified using outside primers²². The $\alpha 1$ homeodomain with mutations G_{108A} and T_{110A} was generated in the same manner, using partially overlapping primers containing the two mutations. The $\alpha 2$ C-terminal fragment was a gift from A. Vershon. All other proteins were overexpressed and purified from *E. coli* strain BL21(DE3) or BL21(DE3)pLysS containing the relevant gene under the control of the T7 promoter in the plasmid pHB40P (ref. 23). Proteins expressed in BL21(DE3)pLysS were induced with 0.4 mM IPTG at $A_{600} = 0.5$ and grown for 5 h; those proteins expressed in BL21 (DE3) were grown without induction. All proteins were purified from cell lysates by adhesion to a cation-exchange resin (Sephadex SP-C50, Pharmacia) followed by elution with a NaCl gradient. The proteins were >90% pure by Coomassie staining following electrophoresis through SDS gels. All operators were designed based on the spacing and orientation of the binding sites in a *bona fide* *hsg* operator⁸. Complementary oligonucleotides were generated for each operator and then annealed to form duplexes with TCGA 5'-overhangs at each end. The oligonucleotides for the *hsg* and the *a1/a1* operators were gifts from C. Goutte.

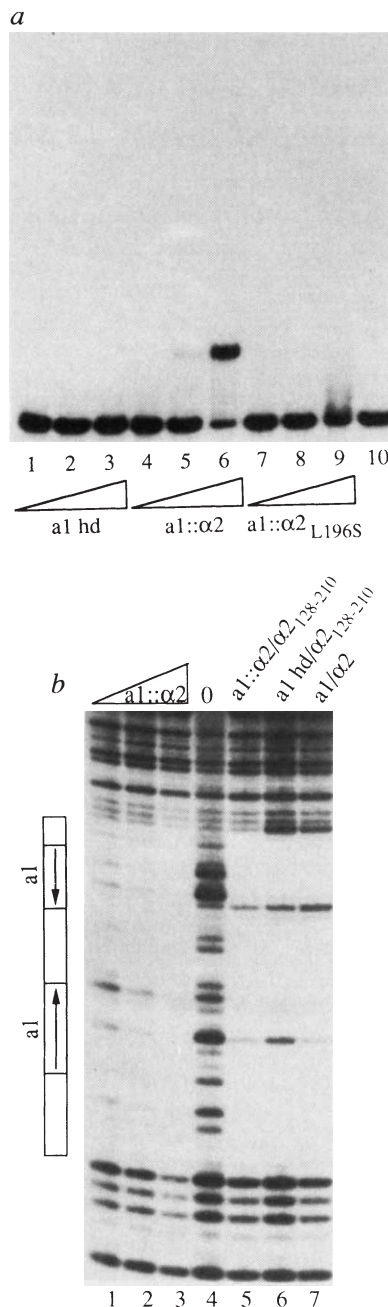
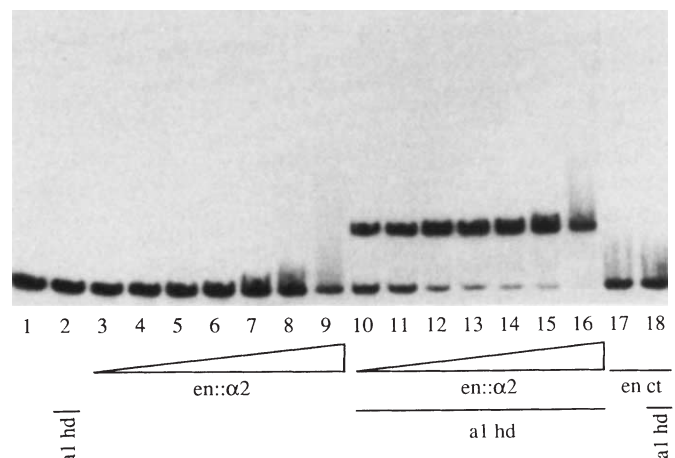


FIG. 2 The *a1* homeodomain:: $\alpha 2$ tail chimaera (*a1::α2*) binds a synthetic *a1/a1* operator. **a**, Electrophoretic mobility shift. A ^{32}P -labelled 80-bp DNA fragment containing two *a1* binding sites (Fig. 1b) was incubated with purified protein. DNA concentration was approximately 10^{-10} M. Lanes: 1–3, successive 3-fold increases of *a1* homeodomain protein beginning with a concentration of 30 nM; 4–6, successive 3-fold increases of *a1::α2* protein beginning with a concentration of 30 nM; 7–9, successive 3-fold increases of *a1::α2_{L196S}* protein beginning with a concentration of 30 nM; 10, labelled DNA alone. The size of the *a1::α2* shift is the same as the shift when both half-sites of the *hsg* operator are filled by two molecules of the $\alpha 2$ C-terminal fragment²⁴. **b**, Protection against DNase I. Labelled *a1/a1* operator was incubated with the proteins as indicated above the lanes of the gel, followed by treatment with DNase I. The two *a1* binding sites of the *a1/a1* operator are indicated to the left of the figure. Lanes: 1–3, successive 2-fold increases of *a1::α2* protein beginning with a concentration of 300 nM; 4, no protein; 5, 300 nM *a1::α2* protein with 100 nM $\alpha 2$ C-terminal fragment; 6, 300 nM *a1* homeodomain protein with 100 nM $\alpha 2$ C-terminal fragment; 7, 300 nM full-length *a1* protein with 100 nM full-length $\alpha 2$ protein. The DNase I footprint of full-length *a1* and $\alpha 2$ proteins has been shown to represent the binding of a heterodimer to the DNA operator⁸. The fact that the *a1::α2* chimaera footprint is nearly identical to the *a1/α2* footprint indicates that there are two molecules of the *a1::α2* protein binding to the operator. In addition, the protection seen with *a1::α2* and the $\alpha 2$ C-terminal fragment together resembles that with *a1* and $\alpha 2$, indicating that the tail of $\alpha 2$ which is attached to the *a1* homeodomain does not interfere with the normal interaction between *a1* and $\alpha 2$.

METHODS. The *a1/a1* operator duplex was cloned into the *Sa*I site of pUC18, excised as an 80-bp fragment, and ^{32}P -end-labelled using Klenow fragment. The DNA-binding experiments were performed as previously described^{4,20}. Protein and DNA were incubated together at room temperature for 45 min in gel shift buffer (5% glycerol, 10 mM Tris-HCl, pH 8, 10 mg ml⁻¹ BSA, 0.1 M EDTA, 10 μg ml⁻¹ non-specific DNA, 5 mM MgCl₂, 0.1% N-P40, 100 mM NaCl). *E. coli* genomic DNA digested with *Hae*III was used as nonspecific DNA. Samples were electrophoresed through a 5% native Tris-borate-EDTA (TBE) polyacrylamide gel for 1 h at 200 V. The DNase I protection experiment was performed as described²⁰, except the buffer used contained 10 mM Tris-HCl, pH 7.0, 5 mM MgCl₂, 10 mM CaCl₂, 0.5 mM EDTA, 50 μg ml⁻¹ BSA, and 2.5 mg ml⁻¹ calf thymus DNA. Reactions were incubated at 20 °C for 1 h before cleavage for 10 min with 1.5 μg DNase I (Worthington). Reactions were stopped and precipitated with 1.6 M ammonium acetate. Samples were electrophoresed through a 10% denaturing TBE gel.

FIG. 3 Cooperative binding of the *a1* homeodomain and the En homeodomain:: $\alpha 2$ tail chimaera (*En::α2*) to a synthetic *a1/en* operator. A ^{32}P -labelled 92-bp DNA fragment containing the *a1/en* operator (Fig. 1b) was used in the electrophoretic mobility shift experiment. DNA concentration was $\sim 10^{-10}$ M, and all proteins were >90% pure. Lanes: 1, labelled DNA alone; 2, 100 nM *a1* homeodomain protein; 3–9, successive 3-fold increases of *En::α2* chimaeric protein beginning with a concentration of 1 nM in lane 3, and ending with 1 μM in lane 9; 10–16, same titration of *En::α2* as in lanes 3–9, but also containing 100 nM *a1* homeodomain protein; 17, 300 nM *En* C-terminal fragment; 18, 300 nM *En* C-terminal fragment with 100 nM *a1* homeodomain protein. The complex equilibrium dissociation constant describing the interaction of *En::α2* and the *a1* homeodomain with the *a1/en* operator is very similar to that observed for the cooperative binding of the C-terminal fragment of $\alpha 2$ and the *a1* homeodomain to the *hsg* operator^{4,9}, $\sim 10^{-16}$ M² as estimated from gel shift experiments.

METHODS. The synthetic *a1/en* operator duplex was cloned into pUC18 and was excised and radioactively labelled as a 92-bp fragment. DNA binding was assayed as for Fig. 2, except that proteins were incubated together at room temperature for 30 min in gel shift buffer containing 5 μg ml⁻¹ nonspecific DNA before addition of the labelled DNA. Protein and DNA were incubated another 45 min at room temperature before being electrophoresed.



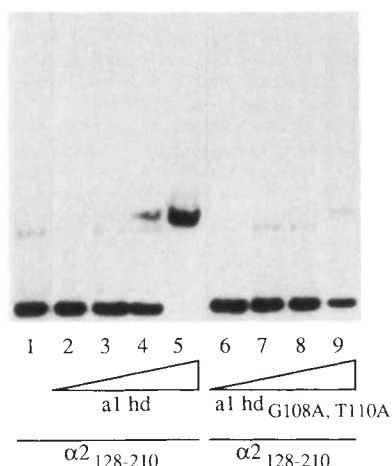


FIG. 4 Specific residues that comprise the turn between helix 2 and helix 3 of the a1 homeodomain are required for cooperative binding with $\alpha 2$. A ^{32}P -labelled 80-bp fragment containing the *hsg* operator (Fig. 1b) was incubated with purified proteins in the electrophoretic mobility shift experiment. Lanes: 1, 3 nM $\alpha 2$ C-terminal fragment; 2–5, 3 nM $\alpha 2$ C-terminal fragment with successive 3-fold increases of a1 homeodomain protein starting at 10 nM; 6–9, 3 nM $\alpha 2$ C-terminal fragment with successive 3-fold increases of the a1 homeodomain protein containing the two point mutations, G108A and T110A, beginning at 10 nM. The two forms of the a1 homeodomain, wild-type and mutant, were expressed at approximately the same levels, and their purification profile was identical. We therefore assume that these changes do not significantly destabilize the a1 homeodomain structure. The DNA-binding experiment is described in Fig. 2 legend.

the adjacent C-terminal 16 amino acids is required for these activities. Some HOM-C proteins from *Drosophila*, for example Antp, Ubx, and Dfd, may also use their C-terminal tails to alter target gene specificity by interacting with other proteins^{16,17}. It is therefore plausible that the C-terminal tails of many homeodomain proteins could prove to be important protein interaction domains which determine protein partners and, as a result, the specific sets of genes to be regulated. □

XPG endonuclease makes the 3' incision in human DNA nucleotide excision repair

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HUMANS with a defect in the XPG protein suffer from xeroderma pigmentosum (XP) resulting from an inability to perform DNA nucleotide excision repair properly^{1–4}. Here we show that XPG makes a structure-specific endonucleolytic incision in a synthetic DNA substrate containing a duplex region and single-stranded arms. One strand of the duplex is cleaved at the border with single-stranded DNA. A cut with the same polarity is also made in a bubble structure, at the 3' side of the centrally unpaired region. Normal cell extracts introduce a nick 3' to a platinum–DNA lesion, but an XP-G cell extract is defective in making this incision. These data show that XPG has a direct role in making one of the incisions required to excise a damaged oligonucleotide, by cleaving 3' to DNA damage during nucleotide excision repair.

The mechanism by which damaged DNA is specifically incised during nucleotide excision repair in eukaryotes is not well understood, but two unrelated nuclease activities seem to be involved. The human XPG protein and its *Saccharomyces cerevisiae* homologue Rad2 are able to cut single-stranded M13 DNA^{5,6}. The *S. cerevisiae* Rad1/Rad10 protein complex also cleaves single-stranded DNA^{7,8}, and a similar activity is anticipated for its human counterpart, the ERCC1/XPF complex^{9–11}. Because of their involvement in nucleotide excision repair, it has been proposed that XPG may incise on one side of a lesion in DNA, and ERCC1/XPF on the other. Single-stranded regions could arise as DNA flanking an adduct is opened by DNA helicases and damage recognition proteins¹².

To test whether XPG cuts at the boundary between double and single-stranded DNA, 'splayed-arm' DNA structures were constructed (Fig. 1a). Substrates A and B have a duplex region of ~30 base pairs (bp) and single-stranded arms of ~30 nucleotides. Cleavage with XPG was tested when the 5' end of one strand was labelled with ^{32}P in each of the four possible combinations. Specific cuts were observed whenever the labelled strand had a 5' single-stranded end (structure A labelled on strand 1, or structure B labelled on strand 3; Fig. 1b, lanes 1 and 5). In both cases the incisions were made at the branch point, a few bases into the duplex region. This junction region may have a partially paired, 'breathing' character. With an amount of XPG 10-fold higher than required to correct the repair defect in an XP-G cell extract⁵, ~15% of the cleavable strands were cut. XPG did not cleave the labelled strand when it had a 3' single-stranded end (structure B labelled on strand 1, or structure A labelled on strand 2; Fig. 1b, lanes 2 and 4). The lack of cutting within the single-stranded regions indicates that XPG does not cut single-stranded DNA indiscriminately. Cleavage was not sequence specific, as the sequences flanking the cuts were different in structures A and B. Furthermore, strand 1 was nicked when it was part of structure A (Fig. 1b, lane 1), but not in structure B (Fig. 1b, lane 2), or when present in fully duplex structure C (Fig. 1b, lane 3). The XPG endonuclease is thus structure specific and displays polarity.

Because of the position of the incision at the branch point in the splayed-arm substrates, XPG was tested for its ability to cleave a 'bubble' structure consisting of a 30-nucleotide unpaired region flanked by duplex regions of 30 bp (Fig. 2a). This may

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more closely resemble the structure which is envisaged to be present during excision repair after unwinding or separating the DNA strands by other repair proteins. XPG incised the labelled strand of the bubble near the 3' end of the unpaired region, ~3 bases into the adjacent double-stranded region (Fig. 2*b*, lane 2). The polarity of cutting was compatible with that seen for the splayed-arm substrates (compare Figs 2*a* and 1*a*), and shows that cutting did not require a free single-stranded 5' end. The

efficiency of cutting of the bubble was ~50%, so it may be a better substrate than the splayed-arm structures. XPG cleaves M13 DNA to produce fragments a few hundred nucleotides long, and this suggests a limited number of preferred cutting sites³. Cleavage of single-stranded M13 DNA by XPG may be structure specific as interactions between complementary regions could lead to the formation of secondary DNA structures related to those studied here.

FIG. 1 XPG cleaves splayed-arm structures with 5' single-stranded tails. *a*, Structures of substrates A, B and C, made by annealing synthetic oligonucleotides with the indicated strand designation numbers. Strong and weak incision sites derived from *b* are indicated by solid and dotted arrows, respectively. *b*, Products obtained after incubation of A, B or C with XPG protein. Lane 1, substrate A labelled on strand 1; lane 2, substrate B labelled on strand 1; lane 3, duplex substrate C labelled on strand 1; lane 4, substrate A labelled on strand 2; lane 5, substrate B labelled on strand 3. Asterisks indicate a 5' label with ³²P.

METHODS. One strand of each substrate was 5' labelled using polynucleotide kinase and [γ -³²P]ATP before annealing with the other strand, and the products were gel purified²⁴. The sequences of the oligonucleotides were: 61-mer strand 1, (5'-GACGCTGCCGAATTCACAGTGCCTTCTAGGACATCTTGGCCACCTGCAGGTTACCC-3'), 62-mer strand 2, (5'-TGGGTGAACCTGCAGGTGGGCAAAGATGTCCATCTGTTGTAATCGTCAAGCTTTATGCCGTT-3'), 62-mer strand 3, (5'-ATCGATAGTCGGATCCTCTAGACAGCTCCATGTAGCAAGGCACTGGTAGAATTCGGCAGCGT-3'), 61-mer strand 4, (5'-TGGGTGAACCTGCAGGTGGGCAAAGATGTCCATAGCAAGGCACTGGTAGAATTCGGCAGCGT-3'). Reaction mixtures (20 μ l) contained 100 ng recombinant XPG (expressed in baculovirus and purified as described⁵) and 0.5 ng substrate DNA in buffer A (25 mM HEPES-KOH, pH 6.8, 10% glycerol, 2 mM MgCl₂, 50 μ g ml⁻¹ BSA, 1 mM DTT). After incubation at 37 °C for 90 min, reactions were stopped by adding 1 μ l 0.5 M EDTA. 20 μ l 90% formamide, 1 mg ml⁻¹ bromophenol blue was added and samples heated to 95 °C for 3 min. Products were separated on a denaturing 12% polyacrylamide gel and visualized by autoradiography or with a phosphorimager. Size markers were made by dideoxy sequencing of M13mp18 using the universal forward sequencing primer.

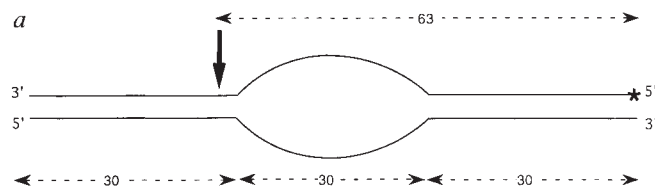
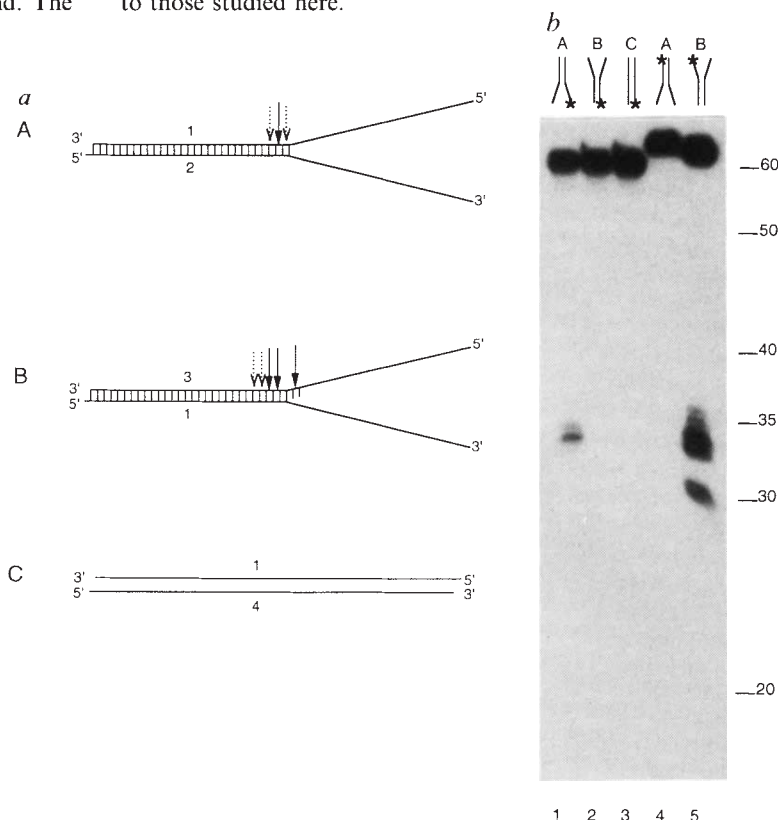
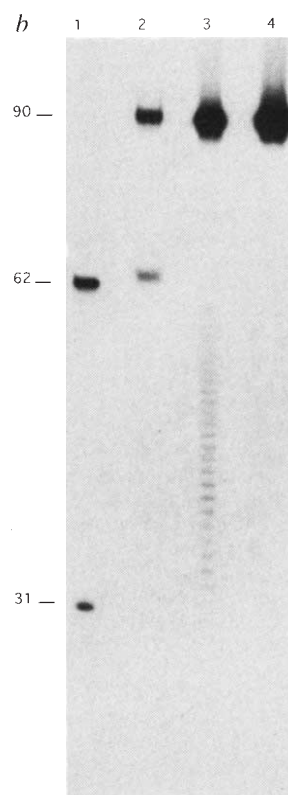


FIG. 2 XPG incises DNA at the 3' side of a bubble. *a*, Substrate formed by annealing two 90-mer oligonucleotides with a centrally unpaired region of 30 nucleotides. One strand was labelled with ³²P at the 5' end, indicated by the asterisk. XPG cleaved the labelled strand at the position indicated by the vertical arrow. *b*, Lane 1, 5' ³²P-labelled marker oligonucleotides; lane 2, products of incubating XPG (100 ng) with 0.5 ng bubble substrate; lane 3, product of a limited digestion of bubble DNA with S1 nuclease, to verify that only the region between 30 and 60 nucleotides was single stranded; lane 4, bubble substrate only.

METHODS. The bubble substrate was gel purified after annealing the 5'-labelled oligonucleotide 5'-CCAGTGATCACATACGCTTGTCTAGGACATCCCCCCCCCCCCCCCCCCCCCCCCAGTGCCACGTTGTATGCCACGTTGACCG-3', to the oligonucleotide 5'-CGGTCAACGTGGGCATACAACGTGGCACTGTTTTTTTTTTTTTTTTTTTTTTTTTTTATGTCCTAGCAAAGCGTATGTGATCACTGG-3'. The XPG endonuclease reaction in lane 2 was carried out as described for Fig. 1. For lanes 3 and 4, bubble DNA (10 ng in a 20 μ l reaction mixture) was incubated for 20 min at 20 °C in 50 mM NaCl, 10 mM ZnSO₄, and 30 mM sodium acetate (pH 4.5), with (lane 3) or without (lane 4) 1 unit of S1 nuclease (BRL).



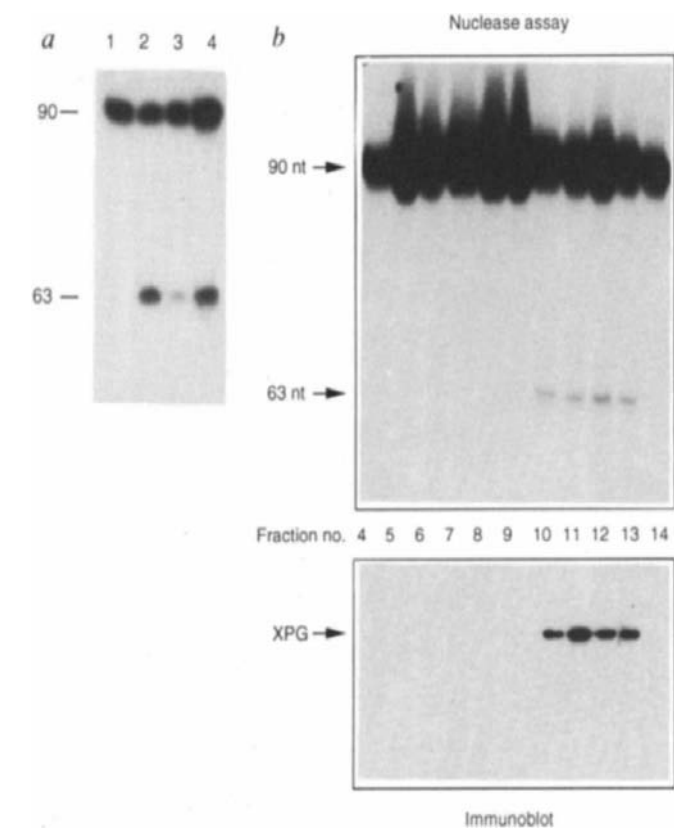


FIG. 3 The nuclease activity is intrinsic to XPG. *a*, Inhibition of nuclease activity by an antibody raised against an amino-terminal polypeptide of recombinant XPG made in *E. coli*^{5,13}. Bubble DNA (0.5 ng, see Fig. 2) was incubated in buffer A alone (lane 1), with 100 ng XPG (lane 2), with 100 ng XPG plus 0.5 μ l immunopurified antibody, an amount titrated to reduce cleavage to 10% (lane 3), or with antibody and 200 ng XPG (lane 4). *b*, Co-elution of nuclease activity (top panel) with XPG detected by immunoblotting (bottom panel).

METHODS. Partially purified XPG protein (10 μ g) in buffer B (25 mM HEPES-KOH pH 7.0, 20% glycerol, 1 mM EDTA, 0.02% NP40, 1 mM DTT) containing 0.1 M KCl, was loaded onto a 0.1 ml column of reactive red 120 agarose (Sigma). The column was eluted with sequential 0.5 ml washes of buffer B containing increasing concentrations of NaCl (0.05 M per fraction). 5 μ l of fractions 4–14 (0.3–0.8 M NaCl) were assayed for nuclease activity on the bubble structure. The same fractions were used to assay for the XPG polypeptide by immunoblotting using a 1/100 dilution of antibody and ECL reagents (Amersham).

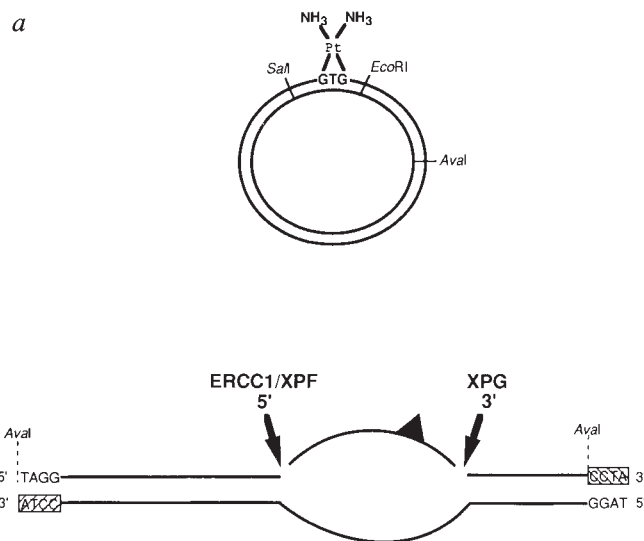
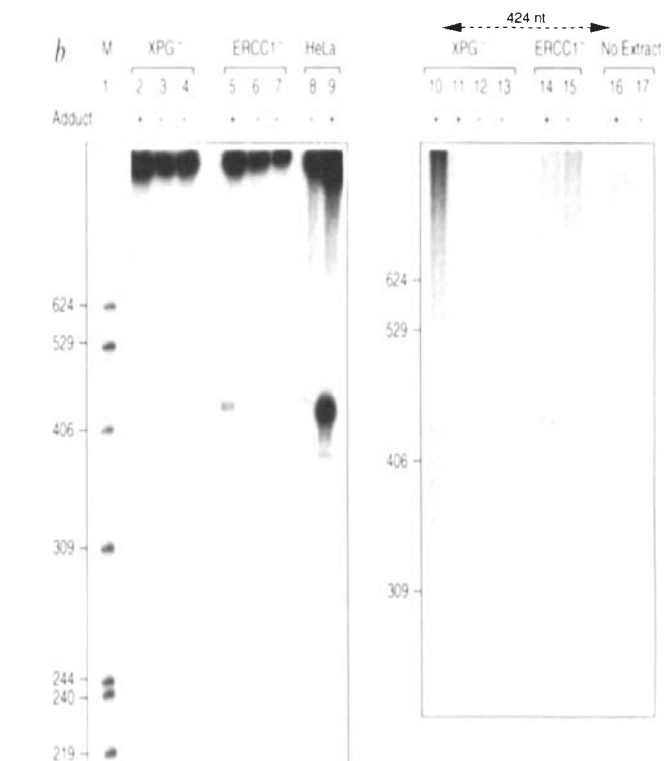


FIG. 4 An XP-G cell extract is defective in making an incision 3' to a cisplatin adduct. *a*, DNA containing a 1,3-intrastrand d(GpTpG) platinum crosslink. The expected positions of the 5' and 3' incisions are indicated. Hatched boxes show dNTPs incorporated during end-labelling. *b*, Detection of incisions formed during nucleotide excision repair. DNA was incubated with cell extracts under conditions that inhibit DNA repair synthesis but permit incision. After cleavage with *Aval*, fragments were 3' end-labelled and separated by gel electrophoresis. An autoradiograph of the gel is shown. Size markers (M, lane 1) are an end-labelled *MspI* digest of pBR322. Substrates with (+) or without (–) an adduct were incubated with XPG-extract (lanes 2–4 and 10–13), ERCC1 extract (lanes 5–7 and 14, 15), HeLa extract (lanes 8, 9), or buffer only (lanes 16–17). Similar results were obtained when 5 μ M aphidicolin was included to further inhibit DNA polymerase (lanes 8–17), or omitted (other lanes). End-labelling used reverse transcriptase (lanes 2, 4, 5, 7, 11 and 13) or DNA polymerase I Klenow fragment (other lanes).

METHODS. *a*, A single 1,3-intrastrand d(GpTpG) cisplatin crosslink in the oligonucleotide 5'-TCTTCTTCTGTGCAC-TCTTCTTCT-3' was incorporated into closed-circular duplex M13mp18GTG DNA with T4 DNA polymerase and ligase following described methods²⁵. A control substrate was synthesized without the adduct. The M13mp18GTG vector was derived by replacing the *EcoRI*–*SalI* fragment of M13mp18 with a 42-bp fragment containing a sequence complementary to the adducted oligonucleotide. *b*, Extracts from CHO 43-3B (ERCC1), XPG415A (XP-G) and HeLaS3 cells were fractionated by phosphocellulose chromatography to make CFII²⁶. 50 μ l repair reactions²⁶ contained CFII protein (50–60 μ g), CFIA protein (24 μ g), DNA (200 ng), buffer without dNTPs, and when aphidicolin (Sigma) was added, 2% DMSO (v/v). DNA was purified, linearized with *Aval* and end-labelled with [α -³²P]dCTP using the Klenow fragment of DNA polymerase I (New England Biolabs) or AMV reverse transcriptase (Boehringer). Purified DNA was separated by electrophoresis on a denaturing 6.5% polyacrylamide gel. A weak band in all lanes migrating just above the incision fragment arises from a small proportion of unligated circles.



The structure-specific endonuclease activity was intrinsic to XPG. An anti-XPG antibody^{5,13} inhibited the activity (Fig. 3a, lanes 2 and 3), and inhibition could be overcome by adding excess XPG (Fig. 3a, lane 4). The nuclease activity also co-purified with XPG polypeptide during chromatography on a reactive red agarose column (Fig. 3b).

Extracts from normal cells can carry out nucleotide excision repair, but XP-G cell extracts are defective in repair synthesis¹³ and excision of a damaged oligonucleotide¹¹. To investigate the ability of an XPG-deficient extract to make the 3' incision, DNA containing a specifically located cisplatin crosslink (Fig. 4a) was incubated with cell extracts. Gap-filling repair synthesis was inhibited by omitting deoxynucleotides from the reaction buffer, but incision could still take place. DNA recovered from the mixture was linearized 424 nucleotides 3' to the lesion (Fig. 4a) and end-labelled. After incubation with HeLa cell extract, a fragment about 420 nucleotides long was detected (Fig. 4b, lane 9). This size is expected from 3' incision roughly five phosphodiester bonds away from a lesion¹⁴. Extract from an XP-G cell line produced little or no detectable damage-dependent incision fragment (Fig. 4b, lanes 2, 10 and 11), whereas some incision fragment was produced by ERCC1-deficient extract (Fig. 4b, lanes 5 and 14).

These data show that XPG protein is required for formation of the 3' incision during nucleotide excision repair. The absence of evidence for a 5' incision by the XP-G cell extract further suggests that either the 3' incision must be made before the 5' incision, or that both incisions are usually made in a coordinated fashion. The ERCC1 gene product may not be absolutely necessary for the 3' incision, but is required for repair synthesis^{9,10} and release of damaged oligonucleotides¹¹. Assuming, by analogy with Rad10, that ERCC1 is part of an endonuclease, we propose that it plays a part in the 5' incision (Fig. 4a).

During nucleotide excision repair in *Escherichia coli*, the binding of a UvrA₂B₁ heterotrimer kinks the DNA and causes local denaturation 3' to the lesion. The UvrB protein then makes the 3' incision at this point, preceding the 5' incision by UvrC¹⁵. This feature of the bacterial strategy may be conserved if the human DNA repair protein complex likewise causes local opening of the DNA, providing a substrate for 3' incision by XPG.

XPG is a member of a protein family that includes *S. cerevisiae* Rad2 and YKL510, *Schizosaccharomyces pombe* rad13 and rad2, and the human rad2 homologue¹⁶⁻²¹. After these studies were completed, we learned that a truncated form of *S. cerevisiae* Rad2 can cleave a splayed-arm structure similar to substrates A and B described here, suggesting that Rad2 may make the 3' incision in *S. cerevisiae*²⁰.

At least two unrelated XPG patients also have features of Cockayne's syndrome²², a disorder associated with suboptimal repair of transcribed regions of DNA²³. During attempted transcription of a damaged DNA strand, the region 3' to a lesion will be the first encountered by the RNA polymerase. As XPG protein acts on this side of the damage, it might be expected to interact with the transcription complex. Some mutations in the XPG gene could affect this interaction, giving rise simultaneously to the DNA repair defect of XP and the transcriptional alterations associated with Cockayne's syndrome.

Note added in proof: Bardwell *et al.*²⁷ have found that the *S. cerevisiae* Rad1/Rad10 protein complex cleaves partially duplex DNA structures near the junction with a 3' single-stranded tail (E. C. Friedberg, personal communication). This suggests that Rad1/Rad10 incises on the 5' side of damage during DNA repair, and strongly supports the proposal that ERCC1/XPF proteins perform this function in mammalian cells. □

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Sp1 elements protect a CpG island from *de novo* methylation

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ANIMAL somatic cell DNA is characterized by a bimodal pattern of methylation: tissue-specific genes are methylated in most cell types whereas housekeeping genes have 5' CpG islands which are constitutively unmethylated^{1,2}. Because methyl moieties derived from the gametes are erased in the morula and early blastula³, this profile must be re-established in every generation; this is apparently accomplished by a wave of non-CpG island *de novo* methylation that occurs at implantation⁴. Using transfection into embryonic stem cells and transgenic mice as a model system, we now show that Sp1 elements play a key role in protecting a CpG island in the adenine phosphoribosyltransferase (APRT) gene from *de novo* methylation. This recognition mechanism represents a critical step in embryogenesis, as it is responsible for setting up the correct genome methylation pattern which, in turn, is involved in regulating basal gene expression in the organism⁵.

Unlike somatic cells, embryonic cells in culture are capable of *de novo* methylating exogenously introduced DNA while at the same time protecting CpG island sequences⁶⁻⁹, and this process clearly mimics what occurs in the normal embryo^{4,10,11}. These same cells can also specifically recognize and demethylate island DNA that has been methylated *in vitro* before transfection⁶. These findings suggest that the bimodal somatic methylation pattern seen in the adult is probably established through the combined action of general *de novo* methylation working

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together with stage-specific factors which either protect island DNA from this reaction or actively demethylate these sequences.

This simple mechanism is probably based on the existence of local *cis*-acting signals that mark each CpG island domain in the genome^{7,12}. These elements were identified by assaying DNA constructs (Fig. 1a, b) for their ability to undergo either *de novo* modification or demethylation following transfection into embryonic stem (ES) cells. Non-island sequences from the hamster APRT gene underwent almost complete modification at *HpaII* and *HhaI* sites following their integration into these cells. In contrast, a DNA fragment carrying the isolated 5' upstream island region remained unmethylated (Fig. 1c). When this same APRT island was transfected into ES cells after *in vitro* methylation at *HpaII* and *HhaI* sites, it underwent substantial demethylation (Fig. 1d). Thus, this DNA fragment must contain all the information necessary for it to be recognized as a CpG island. On further subdivision, several 200-base-pair (bp) DNA segments also proved to be good substrates for this reaction, but some of the smaller 100-bp fragments failed to undergo demethylation. These results strongly suggest that island demodification is directed by discrete *cis*-acting elements and not merely by the high density of CpG residues in these regions (Fig. 2a).

Sp1 elements are frequently found upstream of housekeeping genes¹³ and could represent a potential candidate for a demethylation signal¹⁴. Furthermore, these sites are preferentially associated with the same APRT island subfragments that undergo demodification in ES cells (see Fig. 2a). To test this possibility further, we focused on one specific APRT island *HpaII* site and evaluated its ability to become demethylated in two different sequence contexts. On one fragment this site is linked to upstream Sp1 elements, whereas on a second construct this same *HpaII* locus is associated with nearby 3' sequences that lack Sp1 motifs. As Fig. 2b shows, this *HpaII* site undergoes demethylation only when linked to the Sp1 elements (compare M226 with M225). Moreover, when point mutations are introduced within the Sp1 consensus sequence itself (M231), this fragment is not identified as an island and the *HpaII* locus thus remains modified. We also generated a small 35-bp oligonucleotide containing nine tandem CpG residues but lacking an Sp1 motif (M230), and this DNA was not recognized by the demethylation machinery. Thus, Sp1 elements seem to provide a signal needed to direct demodification.

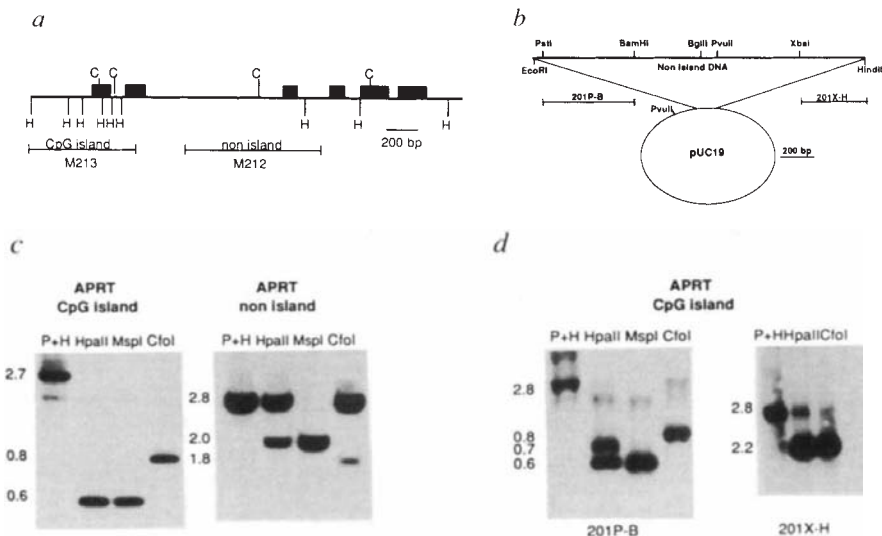
To prove that Sp1 sequences can indeed induce local demethylation, we inserted this element near a *HpaII* site in a non-island region of DNA. The human β -globin gene locus used for this experiment is not only modified *in vivo*¹⁵, but has already been shown to undergo *de novo* methylation when introduced exogenously into cultured embryonic cells¹⁶. Although the *in vitro* methylated wild-type β -globin construct remains modified following transfection into ES cells, the insertion of Sp1 consensus elements¹⁷ near the *HpaII* site leads to a dramatic demethylation at this locus (Fig. 3a). Demodification could be induced within a local range of about 100 bp (Fig. 3b) on either side of the element (data not shown). It is likely that protein factors capable of identifying these Sp1-like elements must also be involved in this process.

To test whether the Sp1 sequence is also a major determinant in the mechanism by which CpG islands are protected from *de novo* methylation in post-implantation embryos, we generated transgenic embryos containing site-directed mutations in all three of the APRT island Sp1 elements (Fig. 4). Unlike the wild-type transgene, which remains completely unmethylated over the entire CpG island region, all the *HpaII* sites in the mutated construct underwent substantial *de novo* methylation. These Sp1 sequences thus seem to be required to prevent local methylation *in vivo*. As Sp1-binding sites are frequently associated with CpG islands in general¹³, it is likely that these sequences are also involved in mediating the undermethylation of other CpG islands in the genome. The human *PGK-1* gene, for example, carries three verified Sp1-binding sites and is completely unmethylated at every CpG residue within its 530-bp island¹⁸. Additional *cis*-acting elements are also probably involved in this process. Not all CpG island regions remain unmodified *in vivo*. Indeed, allele-specific island modification has been found associated with imprinted genes, or on the inactive X chromosome¹⁹. This methylation, however, is apparently part of a separate regulatory process which takes place independently of the major wave of *de novo* modification that occurs around the time of implantation. In these cases methyl moieties are actually added to the DNA later in development^{20,21}.

Although Sp1 sequences are intimately involved in transcription, normal promoter activity is not an essential requirement for demethylation (Fig. 2), and this is consistent with the fact that CpG islands at the 5' ends of tissue-specific genes are consti-

FIG. 1 *De novo* methylation and demethylation of transfected DNA sequences in ES cells. a, Map of the hamster APRT gene including the exons (black boxes), and *CfoI* (C) and *HpaII* (H) restriction sites. *CfoI* is an isoschizomer of *HhaI* (GCGC). b, M201 vector. c, *De novo* methylation. d, Demethylation.

METHODS. b, Fragments from the APRT gene were generated by PCR or by restriction digestion, introduced into the M201 vector, and stably transfected into ES cells. This vector was constructed by inserting an *EcoRI*/*HindIII* fragment derived from an APRT-containing phage²³ into pUC19. This non-island sequence was chosen so as to buffer the test fragment DNA from possible effects of the CpG island-like pUC DNA. c, Constructs were introduced into cells by cationic liposome-mediated co-transfection²⁴ with the L32 *neo* plasmid. Colonies were selected by treatment with G418, pooled and grown to confluence. All DNA samples were digested with *PstI*/*HindIII* alone or with *HpaII*, *CfoI* (*HhaI*) or *MspI*, electrophoresed, blotted and hybridized with the 201P-B probe from the vector (b). The CpG island fragment (M213) encompasses nucleotides 0–710 (GBRO: MAAPRTG) and the non-island region (M212) includes nucleotides 996–1,800. Both were inserted into M201 at the *Bam*HI site. The extreme 5' *HpaII* site in M213 actually consists of two closely linked loci. d, The construct containing the APRT island was first methylated



in vitro with *HpaII* and *HhaI* methylases before transfection into ES cells and then analysed as in c. Hybridization with the 201P-B probe indicates that the most 5' *HpaII* sites are partially methylated, but the adjacent *HpaII* and *CfoI* sites are largely unmodified. Hybridization with the 201X-H probe shows that the *HpaII* and *CfoI* sites at the 3' end of M213 are also unmethylated.

FIG. 2 *Cis*-acting elements required for demethylation in ES cells.

a, Hamster APRT CpG island and constructs used for demethylation analysis. The sequences included in each construct are as follows: M215: 180–430; M211: 467–710; M222: 0–1000; M223: 168–286; and M218: 295–435. The map indicates the positions of the transcription start site and consensus Sp1 elements. M218 was analysed using only *HhaI*, as the *HpaII* site in this region is known to undergo partial demethylation in a non-cell-type-specific manner⁶. b, Role of Sp1 elements in demethylation. On each construct, the location of the CpG residues is marked by a line above the sequence. The nucleotides that were modified in the mutated construct are indicated by a line below the sequence. Scanning of these blots indicated that M231, M225 and M230 underwent about 10% demethylation. In three different transfection experiments M226 underwent 60–80% demethylation.

METHODS. a, The 0.7-kb CpG island portion of the APRT gene, as well as additional smaller fragments, were prepared by specific PCR primers extended with *Bam*HI sites and cloned individually into the M201 vector. Each was then methylated *in vitro* with *HpaII* and *HhaI* methylases, transfected into ES cells and analysed as described in Fig. 1 legend. All constructs were digested with *PstI*/*HindIII* and probed with the 201P-B fragment. b, Small oligonucleotides with overhanging *Bam*HI sticky ends were synthesized, inserted into the M201 vector, methylated *in vitro* with *HpaII* and *HhaI* methylases, transfected into ES cells and then analysed for demethylation after digestion of the DNA with *PvuII* (see Fig. 1 legend). Constructs M225 and M226 carry the overlapping *HpaII* site located in fragment M223. They contain the same number of CpG residues. Construct M231 was derived by mutating the Sp1 sites in M226. The most 5' mutation in M231 was inserted so as to retain an equal number of CpG residues. Using a gel-retardation assay¹⁴, we have shown that oligonucleotide M226, but not M225, M230 or M231, was able to compete for Sp1 binding (data not shown). Construct M230 contains an artificial sequence which includes 9 CpG residues and a *HpaII* site for analysing demethylation.

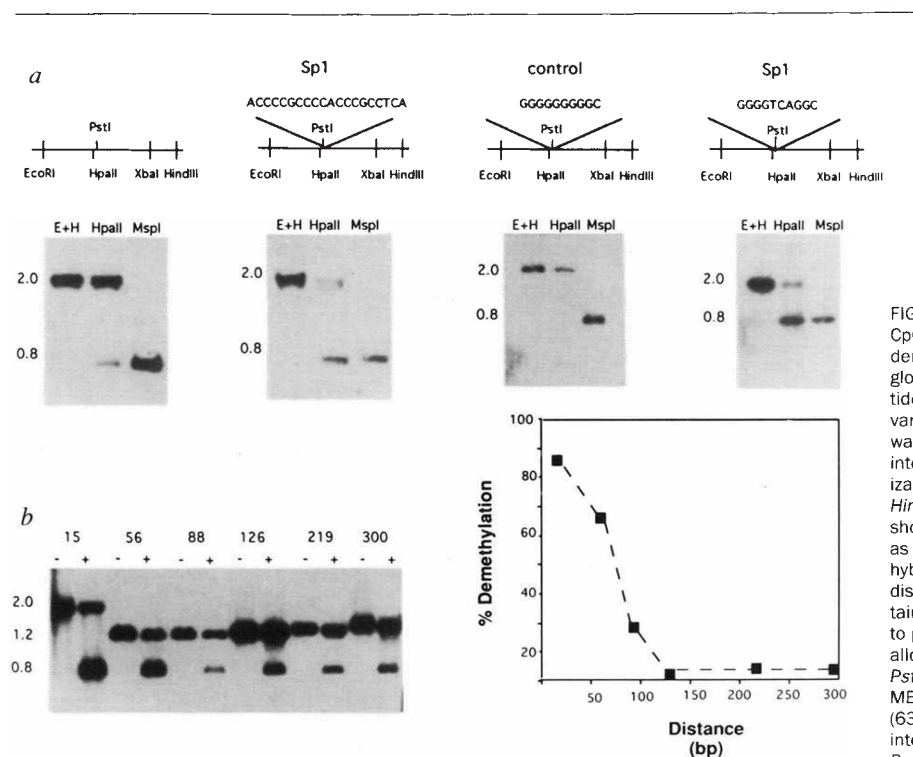
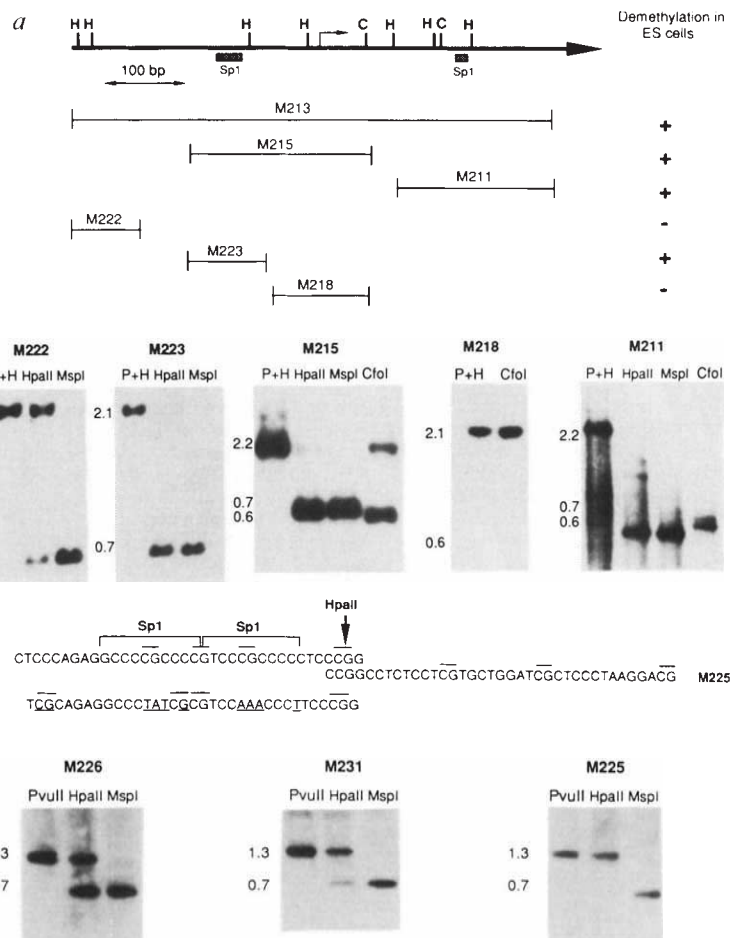


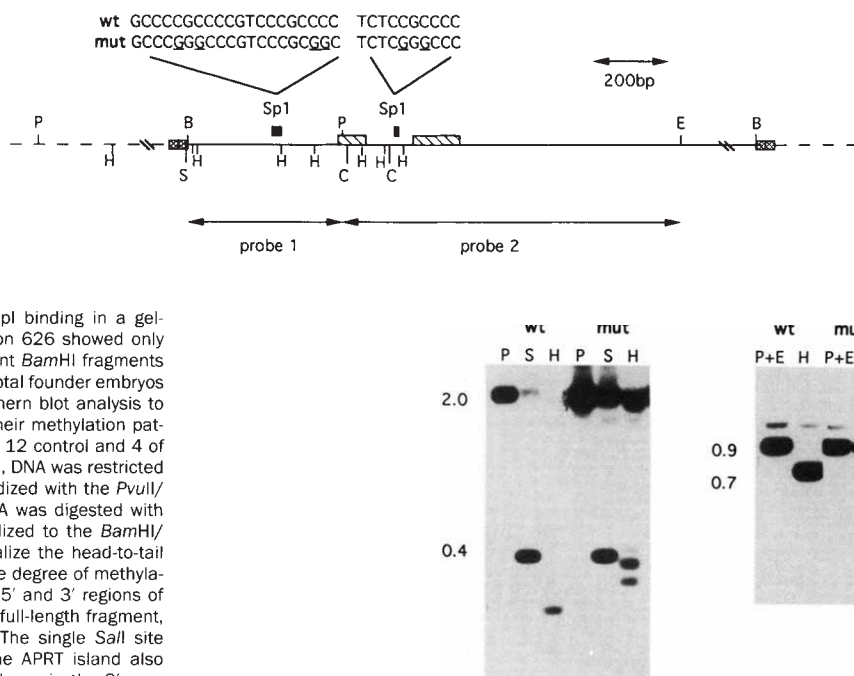
FIG. 3 Sp1 sequences direct demethylation at non-island CpG sites. a, The effect of artificial Sp1 elements on the demethylation of a *HpaII* site located 3' to the human β -globin gene was tested. b, The Sp-1 containing oligonucleotide (ACCCCGCCCCACCCGCTCA) was cloned into M234 at variable distances from the *HpaII* site, and each construct was treated with the *HpaII* methylase *in vitro*, transfected into ES cells and assayed for demethylation by blot hybridization with the *EcoRI*-*PstI* β -globin probe following *EcoRI*/*HindIII* digestion with (+) or without (-) *HpaII*. The graph shows the effect of distance on the degree of demethylation as determined by phosphorimage analysis following blot hybridization. The Sp1 elements were placed at variable distances by generating PCR fragments from the Sp1-containing vector using primers from the 5' end of the Sp1 site to points 3' of the *PstI* locus with *NsiI* and *XbaI* linkers that allowed them to be recombined into M234 after cutting with *PstI*/*XbaI*.

METHODS. The 1.9-kb *EcoRI*/*XbaI* fragment from this gene (63,550–65,437 in sequence Gb:PR:J00179) was cloned into the *EcoRI*/*XbaI* sites of M201 and then digested with *PstI* and religated so as to remove the 250 bp between the two closely spaced *PstI* sites in this construct (M234).

Artificial oligonucleotides carrying an Sp1 sequence (ACCCCGCCCCACCCGCTCA)¹⁷, (GGGTCAAGC)²⁵ or a control sequence (GGGGGGGGC) were then inserted into the single *PstI* site positioned 9 bp from the *HpaII* locus. *In vitro* *HpaII*-methylated constructs were transfected into ES cells and their methylation state was analysed by digestion with *EcoRI*/*HindIII*, blotting and hybridization using an *EcoRI*-*PstI* fragment from the β -globin gene. The degree of demethylation for each construct was measured by scanning and found to be about 80% for those containing Sp1 elements and 10% for those that do not.

FIG. 4 Sp1 elements protect the APRT CpG island from *de novo* methylation *in vivo*. The diagram shows a map of the APRT transgene in the head-to-tail configuration. Restriction sites for *Hpa*II (H), *Cfo*I (C), *Pvu*II (P), *Bam*HI (B), *Eco*RI (E) and *Sal*I (S) are indicated. The locations and sequences of the Sp1 sites that were mutated in this experiment are also shown. The larger hatched boxes mark the positions of the APRT exons and the smaller boxes show the polylinker of the pUC19 vector. wt, Wild type; mut, mutant.

METHODS. Using a pUC19 construct containing the 3.8-kb *aprt* *Bam*HI genomic fragment²⁴, the three Sp1 elements at positions 229, 239 and 558 were mutated by modifying two bases at each site using the Clontech kit²⁶. All these wild-type sequences were able to compete for Sp1 binding in a gel-retardation assay. In contrast, a potential Sp1 site at position 626 showed only weak competition (data not shown). The wild-type and mutant *Bam*HI fragments were then used to generate transgenic animals²⁷. DNA from total founder embryos (14 days post-coitum) was extracted and subjected to Southern blot analysis to detect the samples containing the transgene and analyse their methylation patterns. The gene construct was found to be integrated in 2 of 12 control and 4 of 16 mutant embryos. To assay the 3' portion of the CpG island, DNA was restricted by *Pvu*II/*Eco*RI either alone or together with *Hpa*II and hybridized with the *Pvu*II/*Eco*RI probe (2). To study the 5' end of the CpG island, DNA was digested with *Pvu*II either alone or together with *Sal*I or *Hpa*II and hybridized to the *Bam*HI/*Pvu*II probe (1). In this latter strategy, it is possible to visualize the head-to-tail recombinations of the construct allowing measurement of the degree of methylation of the most 5' CpG island sites. Note that for both the 5' and 3' regions of the island, multiple modifications are required to obtain the full-length fragment, suggesting that each individual site is highly methylated. The single *Sal*I site located in the pUC19 polylinker immediately adjacent to the APRT island also underwent methylation in the mutant construct. The *Hpa*II locus in the 3' non-island end of the APRT *Bam*HI fragment apparently underwent *de novo* methylation in both constructs. CpG island *de novo* methylation was also observed in two other mutant embryos. In contrast, this island was unmodified in two control



embryos from this experiment and was previously shown to remain unmethylated in three founder mice carrying this same APRT transgene.

tively unmethylated *in vivo* although transcriptionally inactive in most cell types¹. The mechanism of CpG island demethylation may be analogous to that involved in the demodification of tissue-specific genes that occurs in individual cell types during organ differentiation. B-cell-specific demethylation of κ -chain sequences, for example, is driven by κ -enhancer-associated *cis*-acting elements in a reaction that occurs independently of transcription and operates regionally over 3–4 kilobase (kb) distances on either side of the inducing sequence²². It thus appears that well known *cis*-acting transcriptional regulatory elements are involved in both stage- and tissue-specific demethylation processes.

Note added in proof: It was recently found that Sp1 elements play a similar role in the mouse APRT gene²⁸. □

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CORRECTION

The mitotic feedback control gene *MAD2* encodes the α -subunit of a prenyltransferase

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WE previously reported that the product of budding yeast spindle assembly checkpoint gene *MAD2* was a component of a geranylgeranyl transferase. Further analysis by our colleague Rey-Huei Chen has shown that the prenyltransferase component is encoded by the gene adjacent to the *MAD2* gene. Thus our conclusion that a prenyltransferase played a role in the spindle assembly checkpoint was in error, although the conclusions that the gene that we had previously identified as *MAD2* encodes the α -subunit of an essential geranylgeranyl transferase, and that this enzyme modifies Ypt1 and Sec4, remain valid. The prenyltransferase-encoding gene has been renamed *BET4* as one of the other subunits of the geranylgeranyl transferase is the product of the previously identified *BET2* gene. The bona fide *MAD2* gene encodes a 196-amino-acid open reading frame (GenBank accession number U14132), which lacks homology to any known genes. □

Uniting the Two Cultures

Walter Gratzer

European Review: Interdisciplinary Journal of the Academia Europaea. Editor-in-chief Arnold Burgen. Wiley. 4/yr. \$195 (institutional); £55, \$85 (personal); £35, \$60 (members).

THE very title conjures up, does it not, the reports of the Sauces and Pickles (Synthetic Additives) Harmonization Commission, or, at best, policy documents on Dairy Farming Subsidies in the coming millennium. I approached the product with misgivings. The inside cover divulges that *European Review* is the organ of the Academia Europa, a body, it seems, dedicated to "bringing together scholars from across Europe, East and West, of all disciplines". What then do they say to each other? Of the eminent editorial board, three out of six are scientists (four if you count the Dismal Science), and they have (judging from the first year's issues) laboured hard to land some big fish.

The first number leads with Heinz Riesenhuber, Minister of Research and Technology for Germany, expounding on the nature and virtues of scientific advisers. In Germany, he tells us, scientific advice goes unpaid and he explains why, to those concerned, this is a privilege, not a misfortune. The minister expatiates approvingly on the activities of the advisory committees that he has created and it is hard to banish from the mind Georges Pompidou's well-known observation that for a politician there are three roads to ruin — women, gambling and taking the advice of experts. Women, he opined were the most pleasant and gambling the quickest, but the last is the surest. Riesenhuber is proud of his Science Council's record in assimilating the government-controlled research apparatus of the GDR into the Federal system and he remarks in passing that the Academies of Agriculture and Building alone employed between them a staff of 36,000. Where, one wonders, are they now? Gone the way, presumably, of most of the 80-odd symphony orchestras and 40 opera houses that a nation of 16 million once boasted. Are they all more gainfully occupied than before?

The Euro-topics in these pages are by no means all without interest. I found impressive an article by the historian Jean-Louis Miège on migrations caused by the displacement of colonialist populations and the problems of absorbing them into a parent culture with which they no longer identify. There is also here an admirable disquisition by the vice-chancellor of Uppsala University on the incursion of industry into the universities. It is hard these days to imagine the following issuing from our British educational *apparatchiks*: "In my view even the most modest university has a mission, and a

responsibility, which goes far beyond the mission of providing industry with efficient employees, marketable ideas or science-based solutions... the production of mature, independent, critical, responsible personalities, who are not tools in the service of either Church, State, party, business or trade unions, is the purpose of the teaching given by these institutions". Scholars, he concludes, will be treated "with respect if they maintain their dignity and uphold their own standards against those of the world at large... and with contempt — and soon enough as simple goods — if they accept without objection the rules of the outside world". More power to him.

There is a notably enjoyable piece by Michael Dummett on the history of card games, and scientists in Britain, if nowhere else, will want to read an engrossing insider's account of how the behemoth, Robert Maxwell, seized control of Pergamon Press and the ruthless adroitness with which he disposed of Paul Rosbaud, the upright German publisher and former physicist, who, under the code-name of The Griffin, had played a heroic part in getting information about the scientific and technological activities

in Nazi Germany to the Allies. Maxwell, of course, knew nothing of science, but (according to one of his early associates) he had made one great discovery — that people could be found who would fill a journal, referee the articles and edit them, all for nothing. We shall (happily) not see his like again.

There is also the odd article in French to keep readers on their toes, but too little evidence of the activities of an English-speaking copy-editor. Something like half the substance of *European Review* is science. The editorial assertion is that articles are to cover "a wide range of topics written so as to make them accessible to readers from other disciplines". But although there are some good pieces — on the science of cork for wine bottles for example, and an absorbing account by A. F. Huxley on the parallel development of ideas in nerve and muscle research — most of it would be taxing for scientists in other fields, let alone onlookers from the outside world. What the average economist, historian or lawyer would make of the dissertations on Ziegler-Natta catalysts or quantum tunnelling is open to speculation, but personally I would doubt whether the narrative will carry them irresistibly along. The fact is that they will read none of it, but at least this may make them uncomfortable, which is perhaps a small step towards a union of the Two Cultures. □

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New Journals review supplement 1994

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (1) the first number appeared during or after June 1992 and at least four separate numbers were issued by the end of April 1994 (although some of the journals eligible for, but not covered in, last year's review issue were also considered)*;
- (2) the journal is published at least three times a year;
- (3) the main language used is English;
- (4) where possible at least four issues should be made available for review, including the first and the most recent numbers.

The time criteria ensure that a reasonable sample of issues is available for judgement by the time reviews are commissioned.

Several journals known to satisfy the criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while some titles were considered to be of marginal interest to *Nature's* audience. Journals covering any aspect of science

were eligible, although those dealing with clinical medicine, engineering and pure mathematics were excluded, as were abstracts publications and newsletters. A list of titles eligible for review but not covered appears on page 458.

The brief given to the reviewers was to limit themselves to comments on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1993 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1994. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rate with the publisher concerned. □

*See *Nature* 365, 569–589 (1993); 359, 435–464 (1992); 353, 457–481 (1991); 347, 581–599 (1990); 341, 350–370 (1989); 335, 459–478 (1988); 329, 357–376 (1987); 323, 359–379 (1986).

Science in society

Jon Turney

Perspectives on Science: Historical, Philosophical, Social. Editor Joseph C. Pitt. University of Chicago Press. 4/yr. \$70 (institutional); \$35 (personal).

Science and Education: Contributions from History, Philosophy and Sociology of Science and Mathematics. Editor Michael R. Matthews. Kluwer. 4/yr. DFL341, \$169 (institutional); DFL162, \$93 (personal).

As the subtitles indicate, both these journals aim to carve new niches in the burgeoning field of science studies. Both are also trying to speak across disciplines. *Perspectives on Science* is for all those pondering how and why science gets done, and how scientific knowledge is validated. The editorial premise is that "a comprehensive and deep understanding of science" requires more interaction between historical, philosophical and sociological studies. *Science and Education* is trying to apply the insights of scholars in science studies to help people teaching real science.

Worthy projects both, and ones that give both journals a great range of subject matter, so obtaining decent contributions is no problem. Not so easy to deliver are genuine interdisciplinarity and a sense of

a coherent project.

Here, *Perspectives*, the newer of the two, is somewhat ahead, although not quite there yet. Many of the (rather long) papers are of general interest, covering such topics as positivism as the organizational myth of science (Stephan Fuchs) or print and early modern science (Henry Lowood and Robin Rider). The ones that are mainly historical often break new ground, as in Allen Harris's yet to be completed account of the Chomskyan revolution in linguistics. There is, though, a dearth of distinctively sociological contributions, despite the presence of several leading sociologists of science among the advisory editors. Do sociologists of scientific knowledge prefer to stick to their own journals, rather than submit papers to one where their fundamental assumptions are occasionally questioned? Or is this just because there are many more historians?

Contributors to *Science and Education* are also mostly dismissive of the stronger claims that knowledge is socially determined. Otherwise, they have less in common than I had hoped. The articles range from lengthy foundation discussions of the cultural meaning of science education to accounts of classroom use of particular historical episodes, with a sprinkling of inaccessible older papers reprinted. As it stands, the journal may have cast its net slightly too wide to develop into a satisfactory package. Lewis Pyenson's reflections

on the history of history of science, for example, are thought-provoking, but would be better placed in a journal that addresses the profession more directly.

At the same time, ironically, it has a limitation often found in discussion of science studies and science teaching. In the four issues offered for review, science is physics. I hope the editor can find some contributors prepared to treat the other sciences. A look at *Perspectives on Science* might suggest some possible authors, if none can be found elsewhere. □

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Après moi le déluge

Arthur M. Lesk

Structure: Form and Function in Modern Biology. Editors Wayne A. Hendrickson and Carl-Ivar Brändén. *Current Biology*. 12/yr. \$504.50, £298 (institutional); \$113.50, £77.95 (personal); \$64.45, £42.95 (student).

THE increasing flow of protein structures has generated a need for new outlets, leading to the expansion of existing journals and the creation of new ones. When creating new ones, editors and publishers can tailor the contents and format to the current state of the field. *Structure* is one of the more attractive newcomers, both physically and intellectually, in the field of structural biology.

Structure publishes several categories of papers. Research articles occupy most of its space, and most of these present new protein structures, determined by X-ray crystallography or nuclear magnetic resonance. A minority of articles contain purely computational analysis or technical methods. Reviews and mini-reviews are sometimes linked to research articles, as in *Nature's* News and Views, but often stand alone. Occasional features are book reviews and a section called "Ways & Means" that discusses technical matters. Georges Cohen has written a "Personal Reflections" piece on β -galactosidase, and others are promised.

A regular feature that is unusual and useful is "Paper Alert", which contains abstracts of articles appearing in other journals and occasionally more extended discussions of a selected one. Each issue features a calendar of meetings. In all, *Structure* is a journal that one can read with interest from cover to cover.

The editors have chosen sensible length limits for articles. Elsewhere, protein structures have sometimes been reported in short letters that whet but do not satisfy the reader's appetite, or in long technical

papers of record that lack emphasis on the biological significance of the work. *Structure* avoids both extremes, and each article includes a section entitled "Biological Implications", appropriately printed in bold type.

Colleagues who have published in *Structure* have been pleased by the mechanism of handling papers. Submission of manuscripts and pictures in computer-readable form is encouraged, and refereeing is prompt, with papers appearing quickly after acceptance. (Of the eight research articles in the 15 May 1994 issue, six were accepted in March and two in April.) There are no page charges, and reproduction of colour illustrations, where justified, is free.

The editors have declared their aim to produce a journal containing papers of high quality and interest, and they have succeeded in this ambition. It does not detract from their success to point out that they are fishing in a well-stocked stream. Indeed, the increased scale of production of good work that has justified the expansion and creation of journals is carrying the seeds of their destruction: first because there is more good work coming out than can fit in a reasonable number of journals of the classical type, and second because it is essential to have access to new structures in computer-readable form.

Structure should therefore be seen in the context of the innovative set of publishing projects of Current Biology. The company's *Current Opinion* journals collect review articles on selected topics; *Macromolecular Structures*, which shares an editor with *Structure*, is an archival atlas; and *Biobase* is a bibliographical database in computer-readable form. These publications, including *Structure*, will be available on the Internet as part of a new service called BioMedNet. Full texts of the articles in *Structure*, including

all figures and tables, will be accompanied by animated graphics.

At present, a nucleus of classical paper journals is surrounded by a sea of electronic databases, newsgroups and other publications (see M. E. Lesk, *Analyt. Chem.* **66**, 747A–755A; 1994). We know by analogy to the periodic table that there are limits to how large the nucleus can get before it falls apart. *Structure* may be one of the last and finest experiments of the *ancien régime*. □

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Yellow Pages for proteins

Roy G. Burns

Protein Profiles. Editor Peter Sheterline. Academic Press. 10/yr. Europe £200, elsewhere \$360; any 5 of 10 issues, £120, \$215; individual issues, £25, \$45.

PROTEIN PROFILES describes itself as a "new kind of journal" that "brings together all available information from sequence and bibliographic data bases in a concise, standardized format". It is more of a review series than a journal, using a common format to tackle specific protein families. Annual revisions of each issue are promised, at least until the authors tire of doing the work for little credit.

Five protein families have had the treatment so far (actin, transcription factors (bZIP proteins), GTP-binding proteins (heterotrimeric G proteins), calcium-binding proteins (EF-hands) and extracellular matrix (fibril-forming collagens)). Each issue has a comparatively brief text, detailed tables that collate the known properties of the proteins, fold-out sequence comparisons and an extensive reference list. How useful are these detailed collations? *Protein Profiles* are like the *Yellow Pages*: both are good for finding out certain specific information, such as the address of a local plumber or whether there is a mutant of a specific amino-acid residue. But just as the *Yellow Pages* does not tell one the name of the best plumber, so *Protein Profiles* does little to evaluate the quality of the reported information. The extensive list of references in each issue (6,854 of them in the issue on calcium-binding proteins) highlights the fact that *Protein Profiles* is a source of raw information that does little to distinguish, unlike most other reviews, between the good, the mediocre and the awful. It will be loved by students who hanker after lists of references that they

should — but probably never will — read.

Much of the more specific information is not readily available from specialist reviews or electronic databases. *Protein Profiles* will be an invaluable source of hard-to-find information on, for example, specific amino-acid mutants or measured ligand-binding constants. The authors should be congratulated for collating the information and for consenting to prepare the annual updates. It is unfortunate that the journal relies on print technology; many potential users would like to have access to the collated data on their computer screens. It is also a pity that the "concise, standardized format" does not always extend to giving complete GenBank – EMBL – Swissprot accession numbers or release dates for all quoted sequences, and that the reference lists lack any details about which databases, keywords or cut-off dates were used in searching the literature. *Protein Profiles* is likely to find a place in most libraries, with experienced workers learning to be careful about how they use the collated information. □

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Microbiological agglomeration

John Postgate

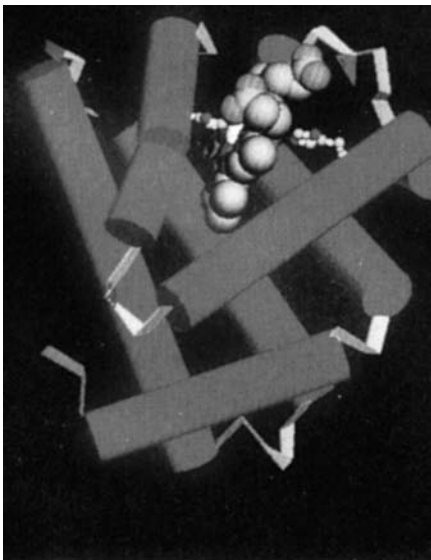
Trends in Microbiology. Editor Caroline Ash. Elsevier. 12/yr. USA and Canada \$490, elsewhere £318 (institutional); USA and Canada \$110, elsewhere £69 (personal); USA and Canada \$55, elsewhere £34 (student).

Medical Microbiology Letters. Editor-in-chief A. von Graevenitz. Birkhäuser. 8/yr. SFr248, DM288, £172.

Microbiology Europe. Editor Andrea J. Sharpe. VCH. 6/yr. SFr106, DM105, \$75, £46 (institutional); free to working microbiologists.

Microbiology. Editor John H. Freer. Society for General Microbiology. 12/yr. USA, Canada and Mexico \$780, Japan £440, elsewhere £420.

WE need a Trades Descriptions Act regarding the titles of scientific books and journals. *Trends in Microbiology* is the fourth misleading title to come my way for review in some 18 months. Its scope is limited to "virulence, infection and pathogenesis" (the subtitle on its cover), but the publicity material gives no hint of this restriction and at least one innocent microbiologist parted with a year's subscription expecting coverage of general microbiology. *Trends in Microbial Patho-*



Arthur Lesk/SPL

Structure of myoglobin as discovered by M. Perutz and J. Kendrew in 1962.

genicity would have been a more honourable title. That said, however, *TIM* is a welcome twelfth in Elsevier's much admired team of *Trends* journals. It follows their now well-established pattern: authoritative reviews (largely commissioned), news, comment, book reviews and an opinion forum, all attractively presented. Articles in the sample volumes deal with, for example, anti-HIV drugs, plant fungal pathogens, antisense RNA in virus therapy, intracellular parasites such as the Chlamydiae, and the pathogenesis of *Helicobacter pylori* — all very topical.

Medical Microbiology Letters finds its niche as a 'quickie' journal that undertakes to print from camera-ready copy, usually within 2 months, either short papers (up to eight pages) or mini-reviews (up to ten pages, although longer ones can

at the National Institute for Biological Standards and Control is perhaps marginally pertinent, but one on river blindness, caused by the nematode *Oncocerca volvulus*, is not microbiology at all. Nevertheless, as a general microbiologist I found something of interest, curious or unexpected, in each of the issues I was sent — and "working microbiologists" (I quote) can receive it free for the bother of filling in a form.

Finally, the *Journal of General Microbiology*, the senior house journal of the Society for General Microbiology and the backbone of British microbiology for nearly half a century, relaunched itself as *Microbiology* at the beginning of the year, with modern presentation, spacious headings and imaginative use of type and boxes. Print, illustrations and paper

Genetic overlap

David Weatherall

European Journal of Human Genetics. Editor-in-chief Giovanni Romeo. Karger. 4/yr. SFr292, \$195 (institutional); SFr204.40, \$136.50 (personal).

Clinical Dysmorphology. Editors Robin Winter, Dian Donnai and Michael Baraitser. Chapman and Hall. 4/yr. Europe £140, USA and Canada \$240, elsewhere £150 (institutional); Europe £55, USA and Canada \$89, elsewhere £55 (personal).

THE field of human genetics, and molecular genetics in particular, is moving so quickly and is so productive that it seems that the market for new journals will remain unsaturated for the foreseeable future. Last year's New Journals supplement featured a complimentary review (*Nature* 365, 569; 1993) of three new journals in human genetics, all with a molecular slant. This year sees two more, both also extremely promising, though, despite the fact that they share an editor, quite different in format and objectives.

The *European Journal of Human Genetics* is the official journal of the European Society of the same name. In the opening leading article the founding editors describe how the journal arose after exhaustive discussions within the society. It was known that several other journals in the field were about to appear and that there were already some excellent ones covering many aspects of human genetics. The editors concluded, however, that "the Society needs its own official voice, which must be a journal of scientific excellence". Brave words in these hard times, but as good a reason as most for embarking on the launch of a new journal. In the leading article that marked the second year of publication, the same editorial board members suggest that the journal should become bi-monthly and increase in size. They apologize for the fact that it will take a year or two to achieve these goals and point out that it is very difficult to move quickly to change a journal that belongs to a society and is therefore subject to the cumbersome processes of democracy.

The editors need not feel too apologetic. The *European Journal of Human Genetics* has managed to get off the mark in this increasingly competitive field with some very solid reviews and many well written and well edited original papers. Although the bias is towards molecular genetics, it already looks as though the journal will encourage a broader coverage of human genetics and welcome a smattering of population genetics; it was a great pleasure to read the opening review by L. L. Cavalli-Sforza and A. Piazza on human genomic diversity in Europe. If the



Mary Evans

Alexander Flemming in his laboratory at St Mary's Hospital Medical School, London.

be published in parts). Layout and print are less than wonderful, but word-processing has improved the appearance of such journals considerably. Within its medical remit the scope is wide: among the letters are reports on laboratory-acquired shigellosis, the flagellar hook of *Borrelia*, the activity ranges of microbicides, and an opportunist *Streptomyces* infection; the two mini-reviews in the samples deal with mycolic acids, part one of a longer article, and with serology in tuberculosis diagnosis.

Microbiology Europe is a relaunch of *European Microbiology*; its first issue includes the fourth part of a series of articles from its predecessor (on occupational infections in health-care workers). Basically it is an advertising medium, emphasizing practical matters, product assessment, laboratory equipment and so on, but it also includes original papers and review articles, together with book reviews and general news about microbiology and biotechnology. The layout and presentation are bitty, with distracting invitations to the reader to comment on articles, or to tick and return requests for product information. The coverage is occasionally odd: a survey of the circular-dichroism facility

quality remain high and "Instructions to Authors" have been revised. The contents still comprise substantial (which means more than preliminary, not necessarily long) papers in basic microbiology (excluding virology), but review papers, long absent from the old journal, have been reinstated. Perhaps its most innovative feature is its "Microbiology Comment" section, which offers microbiologists an opportunity to discuss papers, or simply to float ideas. Thought-provoking items in this section include doubts about the validities of accepted estimates of bacterial volumes, similar doubts about plasmid sizes, and a brief debate on ploidy in *Bacillus subtilis*. The editor's promise of a "more flexible attitude to variations in style and expression" may alarm older microbiologists, who know how easily ambiguous effusions creep into the literature, but the persistence of a hortatory quotation proscribing verbosity at the head of "Instructions" ought to ease our minds. □

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journal can maintain its present standard of original material, which compares well with at least two of last year's newcomers, and continues to present a broader view of human genetics than some of its competitors, it should do well.

Clinical Dysmorphology differs from most of the other journals that cover human genetics. Although some accept a few papers on developmental abnormalities, this new journal aims to devote itself entirely to publishing reports of multiple congenital anomaly syndromes, together with review articles on the aetiology, clinical definition, genetic mapping and molecular embryology of birth defects.

Until recently, clinical dysmorphology was the Cinderella of clinical medicine and human genetics, and even regarded by some as a rather exotic form of stamp collecting. But not any more. The accurate classification of syndromes in dysmorphology is now an absolutely vital prerequisite to studying the molecular basis of defective fetal development. Many of these conditions, rare though they are, have a clear-cut genetic component. The identification of the gene(s) involved, backed up by studies of the genetic and molecular basis of the many mouse models of the dysmorphology syndromes, is a growing and exciting field.

The early numbers of *Clinical Dysmorphology* contain a wide variety of well illustrated papers, most of which, inevitably at this early stage, are largely descriptive and comparative. Hence the stated aims of the journal have been met, but only in part. It may be a while before it starts to turn molecular like its fellow journals in human genetics. But this extremely important field has great potential for human developmental biology; it looks as though this journal may have been born at just the right time.

So journal publishing in human genetics appears to be in fine fettle. There is only one cloud on the horizon. As is clear from the lists of editorial and advisory boards of these journals, the same names crop up again and again. It will become increasingly difficult for new journals in this field to obtain invigorating editorial help. But if they are to remain exciting and vital, as well as evolve a style that distinguishes them from the rest of the pack, some of them will have to start to look for younger and less worn names on their covers.

In case any geneticists take this personally, let me hasten to add that these fears were engendered mainly by my discovery that unbeknown to (or, more hopefully, forgotten by) me, I am a member of the editorial boards of several journals of this genre. □

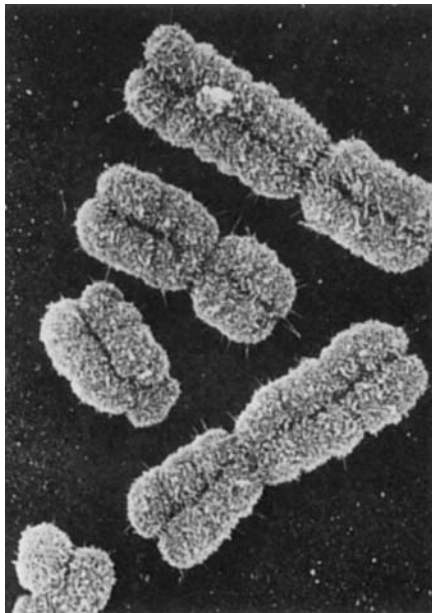
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Different strands

Joy Delhanty

Chromosome Research: An International Journal on the Molecular, Supramolecular and Evolutionary Aspects of Chromosome Biology. Editor-in-chief Herbert Macgregor. *Rapid Communications of Oxford*. 6/yr. £182, \$310 (institutional); £90, \$152 (personal).

In a leading article in its inaugural issue (May 1993), Herbert Macgregor justified the need for this new journal by mentioning that in the previous year there were



Human chromosomes (SEM).

nearly 7,000 articles on some aspect of chromosome biology scattered among 627 different journals, with room for less than 10 per cent of them in journals welcoming papers on chromosomes or cytogenetics. It is true that chromosome research is in an expansive phase because of the technological revolution of the past decade that has seen the development of confocal microscopy, fluorescence *in situ* hybridization (FISH) and associated image capture-and-analysis systems. These developments alone justify a new specialist publication.

The journal intends to cover a wide field of investigation into the evolutionary, supramolecular and molecular aspects of chromosome biology and its scope most closely matches that of *Chromosoma* among existing journals (*Cytogenetics* and *Cell Genetics* devotes the lion's share of its space to human gene mapping).

The initial aim of the editors of *Chromosome Research* was publication within 3 months of receipt, not counting revision time; in the current volume it is working out as 3 to 6 months after revision, but the referees seem to be fairly speedy. The

journal publishes mostly medium length papers (10–15 printed pages), with occasional short communications and technical reports and the odd book review.

Both the academic and technical quality of the papers are generally high, with a recent issue covering topics as divergent as "Chromosome variation in NW American newts", "Conservation and evolution of centromeric satellite DNA in deer" and "The physical map of a 2Mb chromosome of the intestinal protozoan parasite *Giardia duodenalis*", said to be the most primitive eukaryote. For subject matter of this nature the production quality is appropriately excellent, including the colour reproduction. The personal subscription rates give reasonable value for money; there are no free reprints (unlike *Chromosoma*), although authors receive a complimentary copy of the issue in which their contribution appears. □

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Start at the beginning

Jonathan Bard

Zygote: The Biology of Gametes and Early Embryos. Editor-in-chief Brain Dale. Cambridge University Press. 4/yr. USA, Canada and Mexico \$148, UK £86.

GONE are the days when developmental biology was a small field whose practitioners all knew one another and whose publication needs were met by a few journals. The current flood of papers in the area has led to developmental work being published throughout the biological literature, with new journals being set up to absorb the torrents. Some accept any developmental work that has a strong molecular component, but the latest, *Zygote*, focuses on the beginnings of embryogenesis across the phyla (in contrast to *Molecular Reproduction and Development*, which concentrates on early mammalian development). This specialization by topic rather than technique is sensible as molecular approaches in this field are now taken for granted.

One test for deciding whether a relatively narrow area needs an additional journal is to ask if the new publication attracts substantial papers. But because good manuscripts can always find a home, there is a second question that should be asked. Do the workers in the field want to communicate with their peers as well as improve their curricula vitae? The criterion here is whether the editor wishes to include, and can attract, more than just research papers.



Eight-week human fetus in amniotic sac.

It is a pleasure to report that, on both counts, *Zygote* scores well. This quarterly contains articles that early embryologists will need to read, and that later ones may find useful. Its format is modern, its plates are well produced and it is fairly priced. Further, early issues contain a commentary or a review, while the editors have instituted a "Forum" where they hope that controversial areas will be discussed. It is still too soon to decide whether the contributors to these features can keep up their early momentum, but, even if they do run out of steam, *Zygote* has made a good start in attracting interesting papers, and will probably become necessary reading for workers in the field.

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Nerve centres

Michael R. Hanley

Clinical Neuroscience. Guest editors. Wiley-Liss. 4/yr. USA \$150, Canada and Mexico \$190, elsewhere \$205 (institutional); USA, Canada and Mexico \$60, elsewhere £80 (personal).

Neurobiology. Editor-in-chief N. Halász. Akadémiai Kiadó, Budapest. 4/yr. \$82.

THESE two journals are the latest entries into the now mature publishing market for neuroscience research. *Clinical Neuroscience* is a review journal, each issue dedicated to a specific theme, whereas *Neurobiology* intends to publish primary research, mixed with reviews and meeting abstracts. *Clinical Neuroscience* describes itself as "a new forum at the interface of the laboratory and clinic". The journal was launched in response to the increasing need for basic scientists to become aware of clinical applicability, and for clinicians to appreciate fundamental disease mechanisms. Existing journals in neurology or psychiatry do not satisfy these needs, because their historical emphasis

has been on case studies and clinical experience, with modest attention to basic neurobiology. But newer journals are starting to bridge the gap between clinicians and basic scientists, albeit in different ways. For comparison, a comparable initiative is the clinically oriented series of *Current Opinion* titles, but these volumes are not dedicated to a single theme, and therefore lack the useful comprehensive quality of *Clinical Neuroscience*.

Clinical Neuroscience uses a format of 8–10 short reviews commissioned by a guest editor on a specialist topic. So far, the journal has drawn topics from the obvious, but useful, menu of specific brain disorders, such as Alzheimer's disease or depression. Although the list of disorders in neurology and psychiatry is long, it will be a challenge to reinvigorate this way of organizing information. The risk here is the endless recycling of neurological diseases, stifling intellectual renewal of a journal of this type. Perhaps selecting topics that span a variety of diseases, such as novel therapeutic strategies or biological processes, will provide the needed change of pace.

On the whole, the journal is successful. The articles are concise, well written and well illustrated. The topics selected by each editor show a worthy balance between dogma and heresy, giving a balanced perception of controversies and ambiguities. This journal is worthwhile for libraries, but it is quite expensive for individuals, particularly as many of the topics are, in fact, fairly heavily reviewed elsewhere. Nevertheless, this venture has an original and useful vision of bridging the communities of basic and applied neuroscience.

Neurobiology, on the other hand, is an eccentric initiative in recommissioning an existing original research journal. The Hungarian Academy of Sciences has converted an earlier failed journal, *Acta Biochimica et Biophysica Hungarica* into an "international multidisciplinary journal in neurosciences". But the international character of the journal is more in the intent than the observance. Virtually all the published articles are from Hungarian laboratories. Moreover, the journal draws heavily from abstracts from Hungarian societies (of physiology and neuroscience, for example), symposia and so on. Overall, the contributions are fairly marginal and of limited interest. There is little more to say — this is a parochial journal, struggling to develop from an exclusively Hungarian base. In view of the stiff and excellent competition for publication of original articles in neurobiology, the journal is unlikely to make much of an impact, and it cannot really be recommended.

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Ins and outs

William A. Catterall

Receptors and Channels. Editor-in-chief E. A. Barnard. Gordon and Breach. 4/vol. ECU382, \$458 (institutional); ECU90, \$108 (personal).

OVER the past decade, research on receptors, ion channels and transporters has flourished owing to a fortunate confluence of improved methods for purification and characterization of complex membrane proteins, convenient methods of cloning their genes and efficient expression systems for analysis of how they work. This flood of important advances has spread across the pages of *Nature* and many other journals. The volume of important work on these key cellular regulators has long justified the introduction of a new specialty journal focusing on their molecular and cellular properties. *Receptors and Channels* made its debut just over a year ago with the aim of filling this niche. It is off to an excellent start.

The senior editors provide a broad view from Europe, North America and Japan and cover the diversity of subject matter in this field with expertise in cellular and molecular biology, biophysics and electrophysiology. The international editorial board contains a wide range of acknowledged experts in the main areas of interest. Early issues contain noteworthy articles on potassium and chloride channels, inositol 1,4,5-trisphosphate receptors, G proteins, protein kinases, the multidrug transporter and a range of cell-surface receptors. As the field continues to expand, we can look to an increasingly important array of articles of interest to cell and molecular biologists, physiologists, pharmacologists and other biomedical researchers.

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"IF ONLY THERE WERE SOME PEACEFUL USES FOR NERVE GAS."

From Stress Test by Sidney Harris (Rutgers Univ. Press).

Giving up and breaking down

Stuart Sutherland

Addiction Research. Editors-in-chief J. B. Davies and E. Drucker. *Gordon and Breach*. 4/yr. ECU198, \$238 (institutional); ECU124, \$90 (personal).

Depression. Editor-in-chief C. B. Nemeroff. *Wiley-Liss*. 6/yr. USA \$150, Canada and Mexico \$210, elsewhere \$232 (institutional); USA, Canada and Mexico \$60, elsewhere \$90 (personal).

WITH refreshing honesty, one of the editors of *Addiction Research* writes: "There are very few instances in the addiction field of stunning new insights". How right he is: our ignorance of both the causes



Mary Evans/1938

claimed. As to causes, unfortunate life events play a role but just how is unclear. Abnormal activity in some neurotransmitter systems is also implicated, but we have no firm knowledge; as one of *Depression's* contributors lamely remarks: "it appears that interactions among norepinephrines, serotonergic and dopaminergic systems may be of paramount importance". Quite so, but why not throw in the glucocorticoids as well? Moreover, the mechanisms by which the specific symptoms and feelings of depression are produced are completely unknown. Like *Addiction Research*, *Depression* covers all aspects of its subject from "Grief Intensity in Late-Life Spousal Bereavement" to "Triated Platelet Imipramine Binding".

Since both journals can and do claim to have that magic quality of being interdisciplinary, they are likely to be bought (although the price of *Depression* is monstrous, particularly as it takes advertisements from drug companies boosting their wares). Many of the articles contain such time-honoured threats to the reader as "The need for further controlled prospective data is highlighted". Unless the data in question are more informative than any in these two journals, one can only hope that their originators will have the decency to keep them to themselves. □

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Unforgettable

Andy Calder

Memory. Editors Susan E. Gathercole and Martin A. Conway. *Lawrence Erlbaum*. 4/yr. Europe £40, USA \$82, elsewhere £43 (institutional); Europe £20, USA \$44, elsewhere £23 (personal).

In 1991 four psychologists at the University of Lancaster, England, organized an international conference on memory. The meeting was attended by prominent researchers in all subdisciplines of memory research and highlighted the breadth of work in this area. Despite this spread, the attendants were pleasantly surprised by the large amount of overlap among the current research approaches. It would seem that as research on different aspects

of memory has progressed, psychologists have borrowed successful theories and techniques from neighbouring disciplines. Any attempt to tie together different branches of such a broad area of study should be encouraged, and to promote this approach two of the conference organizers, Gathercole and Conway, have provided psychologists with a forum in which to present high-quality research on all aspects of memory.

Succinctly titled, *Memory* has already provided subscribers with a steady flow of good papers and is excellent value for money; better value still for those interested in one of the topics covered in the series of special issues, which have so far addressed "Memory tests and techniques" and "Memory for proper names". The latter issue is particularly interesting and includes four important neuropsychological case studies. These report cases of brain-injured patients who showed a preserved ability to recall proper names (for instance, 'John Major' or 'London') along with impaired recall of common names (for example, 'telephone' or 'stethoscope'). Before the publication of this special issue, accounts of this particular pattern of performance have been extremely uncommon, whereas there have been numerous reports of patients showing the reverse pattern. Until now, psychologists have taken this marked asymmetry of selective breakdown into account when developing models of naming. These new studies suggest that the mechanisms underlying recall of proper and common names can be dissociated, posing a considerable challenge to current models of naming.

The editors state that one of their policies is to produce special issues on topics of exceptional current interest and on research perspectives with which the general readership will not be familiar. With two more special issues already planned ("Long-term retention of infant memories" and "Semantic memory and semantic representations") this feature seems destined to become an important aspect of the journal's character.

Clearly *Memory* is not intended to provide a home for an emerging research discipline. Instead it provides a forum for psychologists interested in all aspects of memory to share their literature reviews, psychological tests, empirical data, computational models and theories. It is easy to forget that a laboratory-based study examining subjects' abilities to remember unfamiliar objects may be tapping similar memory systems to an eyewitness testimony interview. *Memory* aims to remind us of this possibility and offers access to a number of perspectives on memory functions and how best to study them. □

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and the mechanisms of addiction remains almost complete, while the only methods of alleviating the condition unequivocally shown to be useful are replacement therapies — methadone for heroin addicts and the patch, the lozenge or the forthcoming nasal spray for smokers. Although the nicotine spray is likely to be the most effective product for weaning people from tobacco, it has been licensed in the United Kingdom only under a doctor's prescription. This is surely bureaucratic folly, for however addicted ex-smokers become to the nicotine spray, it must be better for them than inhaling tar and carbon monoxide. Indeed, it is not yet proven that small doses of nicotine have any adverse effects.

Addiction Research loftily ignores such down-to-earth issues. Most of its pages are devoted to alcoholism: perhaps appropriately, there is a high proportion of Finns among the authors. Many of the articles are vacuous. One of them concludes that people drink more if they are with friends who are drinking: it disguises and dignifies this banal finding by referring to friends as "the social context".

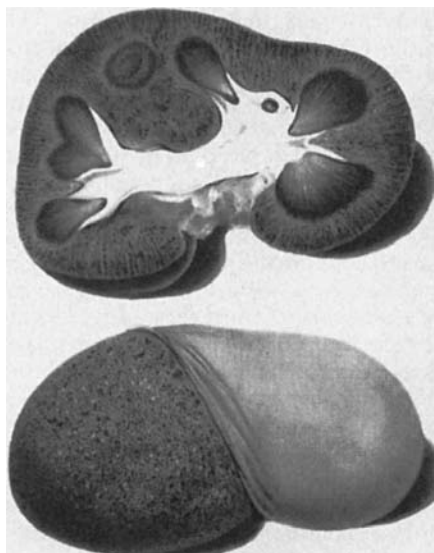
We know a little more about depression than about addiction, but the gaps in our knowledge are much wider than is usually admitted. True, cognitive therapy and antidepressants are some help but their effects are considerably less than is often

Tubular tales

A. S. Woolf

Experimental Nephrology: European Journal of Renal Research. Editor-in-chief L. G. Fine. Karger. 6/yr. SFr594, \$397 (institutional); SFr267, \$178.70 (personal).

NEPHROLOGY has ancient roots in the tasting of urine and proceeded by way of morbid anatomy to physiology. More recently, clinicians have attempted to understand common inflammatory diseases such as nephrotic syndrome, in



Engraving of human kidney (c.1830).

which massive amounts of albumin leak into the urine. Most advances in this area of immunology were, however, fortuitous and we still have no real idea why corticosteroids are effective in treating the syndrome. In retrospect, the physicians could have used a little help from basic scientists. Today's era of renal research, coinciding with advances in molecular and cell biology, has finally married the disciplines. This year, a mutation that causes multiple renal cysts and kidney failure, one of the commonest inherited diseases in humans, was isolated after a decade of collaboration between clinicians and scientists.

The editor of *Experimental Nephrology* hopes that the journal will be a forum for European clinicians and scientists who want to understand the mechanisms of kidney diseases. Certainly, a journal devoted to the basic biology of kidney disorders is now needed more than ever, not only to disperse new information from cell and molecular studies but also to counteract the plethora of descriptive clinical reports to be found in established nephrology periodicals. Whether this type of research really needs a uniquely European perspective is debatable, and I hope this editorial angle does not prevent the

flow of articles from the rest of the world.

At present, the best features of the journal are the excellent reviews and, so far, the original research articles have been good, although not of the highest standard. Important areas addressed have included transplantation tolerance and the biology and potential therapeutic uses of renal cytokines. Gene therapy, a major challenge in a solid organ like the kidney, has also been highlighted. Recent advances in understanding the genetic basis of renal diseases have not yet been featured. The layout is user-friendly (there are plenty of illustrations, often in colour) and the time between acceptance of articles and publication is reasonable. This is a journal that should be supported on a worldwide basis, not just European. □

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Hormone cocktail

Jamshed R. Tata

Endocrine. Editors P. Michael Conn and Ben Greenstein. Macmillan (UK). 12/yr. £265 (institutional); £99 (personal).

THERE was a time when endocrinology was a self-contained discipline, often largely confined to the physiology and biochemistry of endocrine organs. The older journals, such as *Endocrinology*, *Journal of Endocrinology* and *Acta Endocrinologica*, reflected this emphasis on the site and mechanisms of hormonogenesis. Not so any more. The science of hormones has found its way into an increasingly wide variety of other disciplines of molecular and cell biology, especially membrane biology, neurobiology, cell signalling and gene expression. It explains the remarkable proliferation in the past 10 to 15 years of specialized journals in which hormone research features prominently, such as *Molecular Endocrinology*, *Molecular and Cellular Endocrinology*, *Journal of Neuroendocrinology*, *Cellular Signalling*, *Journal of Receptor Research*, *Neuroendocrinology* and so on. Does the launch of *Endocrine* (called *Endocrine Journal* in its first year) represent then a reversal of the trend towards specialist hormone-oriented journals?

Any reversal in *Endocrine* is more apparent than real. Almost all the topics treated in the first year's issues are covered in the above specialized hormone-orientated journals. For example, 15 original papers in the June 1994 issue deal with receptor gene expression, extracellular matrix, mitogenic response to growth factors, neuroendocrinology, receptor localization, developmental endocrinology, hormone metabolism and intracellu-

lar signalling. So there is something for everyone with an interest in the many different aspects of hormonal signalling, a fact also reflected in the make-up of the international editorial board. Who then will benefit from *Endocrine*? It is unlikely to be the average reader of the traditional endocrinological journals. In their foreword to the first issue of *Endocrine*, the editors state that they intend to publish papers "on the periphery of endocrinology" as well as "clinical pages with a basic science message". This is certainly true for the first statement, but so far there is not much evidence of the latter.

Apart from original papers, *Endocrine* also publishes reviews which are of a generally high quality. A unique feature is the appearance from time to time of "Legends in Profile", written by V. C. Medvei. These are brief biographical notes on biologists and clinicians (including such giants as L. Spallanzani, R. de Graaf and T. Addison) who, directly or indirectly, laid the foundations of modern endocrinology. Graduate students should be made to read these. The editors promise that the journal will be "author-orientated", which means that they aim to process and publish manuscripts rapidly. The format and appearance of the journal are attractive, although some micrographs and *in situ* hybridizations would have benefited from colour printing. If the editors and publishers can sustain the present speed and quality of publication, and introduce a cheaper personal subscription rate, then the broad coverage of hormones should ensure that *Endocrine* has a postnatal period of healthy growth and development. □

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Good reception

R. C. Hughes

Cell Adhesion and Communication Editors-in-chief Clayton A. Buck and Jean-Paul Thiery. Gordon and Breach. 4/yr. ECU572, \$687 (institutional); ECU124, \$149 (personal).

CELL ADHESION AND COMMUNICATION resembles most closely in appearance and content *Molecular Biology of the Cell* (formerly *Cell Regulation*) and *Cell Motility and the Cytoskeleton*, both of which are well established outlets for papers dealing with receptor-based cellular interactions and cell signalling. There is a close similarity in content too with a large and increasing part of the two main journals of cell biology, *Journal of Cell Science* and *Journal of Cell Biology*. Most of the excit-

ing new aspects of molecular cell biology are within the stated scope of the new journal, including cell-cell and cell-matrix adhesive mechanisms together with downstream signalling pathways as determinants of gene expression and morphogenesis, molecular mechanisms of cellular responses to cytokines and chemotactic factors, intracellular transport and cellular interactions in immune responses. With the huge popularity of these topics, there is inevitably a niche for good quality journals to relieve pressure on the principal cell-biology outlets.

Cell Adhesion and Communication seems likely to succeed in filling part of this niche along with other good secondary journals. The 29 papers in the four issues of the first volume are generally of high quality and interest and indicate a nice geographical spread of contributors, reflecting presumably the well balanced make-up of the editorial board. The quality of the illustrations is good and it is to be hoped that in time the publishers will find a way to place the (free) colour pictures in context, without segregating them at the back of each issue. The cost of the journal appears to be fluid, with the personal subscription rate rising by 50 per cent in 1993 to \$135 — a bit steep at the present slimline stage of evolution. The publishers are coy about academic and corporate institutional rates but, if reasonable, *Cell Adhesion and Communication* will be a valuable addition to the library shelf. □

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Drugs, cells and mechanisms

N. G. Bowery

Cellular Pharmacology. Editors Claude Jasmin, Kenneth D. Tew and Nagahiro Saijo. *Macmillan (UK)*. 6/yr. Europe £125, elsewhere £135.

PHARMACOLOGICAL research embraces a variety of disciplines from molecular biology to behavioural psychology. So, not surprisingly, there are many journals already devoted to the field, and one wonders whether there is any need for a new one. The editors and presumably the publishers of *Cellular Pharmacology* obviously think so.

In fact, the editors have addressed this question in a brief leading article in the opening issue. They believe that the journal will provide a niche for "work which is not directly defined by the review criteria of existing journals" and go on to say that "many existing journals return without review those papers that meld the disciplines of cellular biochemistry and pharmacology". For their sakes I hope they are correct.

There are, however, many specialist journals that already bridge this divide. *Molecular Pharmacology*, *Biochemical Pharmacology* and even more general pharmacology journals such as *British Journal of Pharmacology* publish papers based on research at a cellular level. So what is the novelty of this new journal?

An obvious attraction would be a short publishing time, which the editors attempt to keep to a minimum by requesting that papers be submitted on disk. Unfortunately, none of those published so far prints the date of submission or acceptance, so there is no indication of speed.

The papers are clearly typeset on good quality paper. The layout is comparable to other journals, although the figures, and particularly the captions, are variable. Articles consists of the usual sections; I fail to see the advantage of describing the methods and materials last, however.

The scope is wide — drug mechanisms at the cellular level — and the editors intend eventually to run up-to-date

reviews. On the whole, I did not find the articles (about eight per issue) particularly exciting, although one did catch my eye: "Interaction of new compounds at microtubular level" — the ultimate in imprecise titles. Reductionism in pharmacological research has become a major trend and, with this in mind, the journal may provide the extra publication space needed for the resulting flow of work. □

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Surface impression

Clare M. Isacke

Epithelial Cell Biology. Editor Christopher S. Potten. *Springer*. 4/yr. £120 (institutional).

SKIN, blood, bone, muscle, liver and many other parts of the body are honoured by their own specialist journals. Should the same be done for epithelial cells and tissues? In my opinion the answer is a definite yes. Workers in the field would benefit greatly from a forum that collects together the reviews, technical advances and original research papers currently scattered throughout an increasingly large number of other publications. Further, there is a genuine need for a journal that encourages papers on a variety of epithelia rather than just the model culture systems. This is undoubtedly a multidisciplinary and flourishing subject area, which produces an inherent problem for a new journal.

Epithelial Cell Biology neither covers a discrete enough subdiscipline to attract a select, specialist clientele, nor aims high enough to do what *Neuron* did for neurobiology: that is, compete for the top-quality publications currently found in the main journals that cover cell biology, development and cancer. It is a journal struggling to maintain a middle ground. Although the quality and presentation of papers are good there are often long delays between their acceptance and publication, and charges are made for reproduction of colour plates.

For now, this slim journal will find it difficult to attract many individual subscribers, but libraries should be encouraged to add it to their racks. I hope it succeeds, although I fear that a firm editorial policy will be required if researchers are to be persuaded to submit their best papers. □

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Ciliated tracheal epithelium (false-colour SEM, $\times 5,000$).

Solid resolve

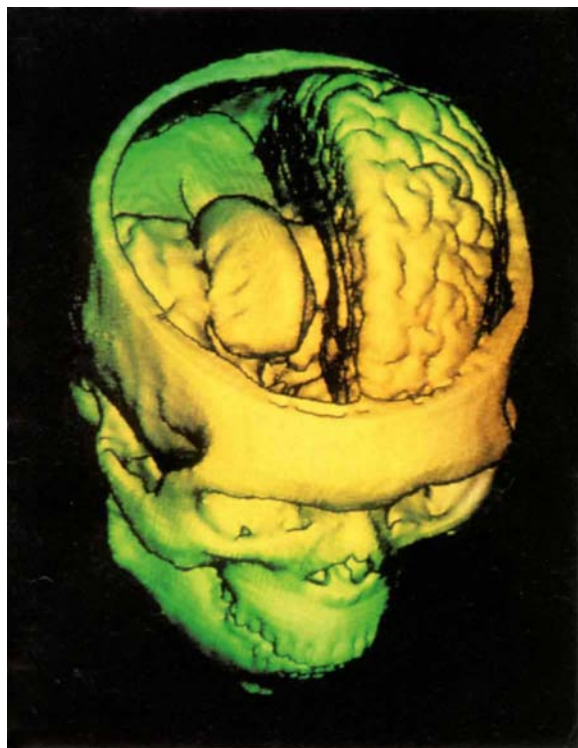
Michael A. Smith

Bioimaging. Editors T. M. Jovin and I. T. Young. *Institute of Physics/American Institute of Physics.* 4/yr. USA, Canada and Mexico \$236 (institutional), \$123 (personal); elsewhere £115 (institutional), £52.50 (personal).

THE field of imaging science has been very productive over the past decade, both in the development of imaging methods and in the analysis of associated images. But it

human magnetic resonance imaging and histology images sat uneasily among the others. Each issue contains about six full papers; these tend to describe original work, although some of the earlier ones have been more in the nature of reviews, much as one would expect in a young journal.

Publication time seems to be rapid, although a brief survey of the two most recent issues reveals that of the ten papers, half are from associate editors or the institutions in which they work. It is not clear to me how the journal will establish a cohesive editorial policy in the long run without an editorial board; for now,



False-colour three-dimensional computer tomography image of the human brain within the skull, showing the right cerebral hemisphere and, on the left, the underlying structures of the limbic system and ventricles. Picture credit: Science Photo Library.

is often the case that the solution to a particular problem is seen in terms of only one of these aspects; there is little crossover between the two. Also, interest has tended to concentrate on what I would refer to as 'macro' imaging, which emphasizes techniques such as computerized tomography and magnetic resonance imaging in humans and other animals. By comparison, 'micro' imaging, which deals with microscopic and cellular techniques, is underrepresented, a situation that needs to be rectified given the continuing rapid growth in molecular science.

The publication of *Bioimaging* is to be welcomed because it focuses primarily on these two interlinked areas of microscopic imaging, in its broadest sense, and on the associated image analysis. In my view, the journal should concentrate on this important niche and not be tempted to broaden its remit as it did in one of the earlier issues: a paper on the registration of

there are two editors and 46 associates.

The format is excellent and the overall quality of production is good, with colour illustrations, where appropriate, throughout. Some of the half-tone illustrations are poorer, though not so bad as to be unacceptable. I suspect that the fault lies with the originals, but, for a journal devoted to imaging, this problem needs to be addressed.

It is often tempting to cry "not another journal!" when a new publication appears. Not so for *Bioimaging*: it has the potential to fulfil a valuable role in providing a forum for specific novel techniques and applications of 'microimaging' that support research in cell biology and medicine. □

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On target

John Woodley

Journal of Drug Targeting. Editors-in-chief A. T. Florence and V. H. L. Lee. *Gordon and Breach.* 4/vol. ECU572, \$687 (institutional); ECU135, \$162 (personal).

DRUG targeting can be traced back to the father of chemotherapy, Paul Ehrlich, who at the end of the nineteenth century was envisaging "substances which possess a high affinity and high lethal potential in relation to the parasite, but a lower toxicity in relation to the body". The emphasis today is on active targeting: the ability, by chemical or biological means, to direct chemotherapeutic agents to specific sites in the body. As a branch of the pharmaceutical sciences, drug targeting has mushroomed in the past 20 years. A major stimulus was the advent of monoclonal antibodies in the 1970s, which promised highly specific targeting to cells, particularly cancerous ones. The 'magic bullet' was becoming a reality. Although the euphoric promise of monoclonal antibodies has failed to materialize, there is now a range of credible systems being investigated for drug targeting, including liposomes, micro- and nano-particles and synthetic polymers, as well as antibodies and antibody fragments. Several of these are being examined in clinical trials, and all of them feature in this new journal.

The appearance of *Journal of Drug Targeting* is therefore timely and welcome as the area of study comes to maturity. It has an excellent format and gets top marks for readability and quality of papers. The submission dates indicate that the editors are achieving fast acceptance times, much to their credit. The editors rightly stress the multidisciplinary approach of drug targeting and delivery. Imaginative research in pharmaceutical sciences will never emanate from the narrow confines of conservative disciplines. The first few issues achieve this multidisciplinary goal, with an encouraging mix of papers from both academic and industrial sectors worldwide.

It is probably inevitable that the first few issues of a new journal are slightly less selective than a well established one. I hope that the editors can keep themselves clearly targeted and limit the journal mainly to papers justified by its title (albeit broadly interpreted). Some early papers are marginal to the journal's objectives and would be better suited to traditional pharmaceutical journals or other specialized journals such as *Journal of Controlled Release*. But overall the new periodical is excellent, covering exciting developments. It deserves to succeed. □

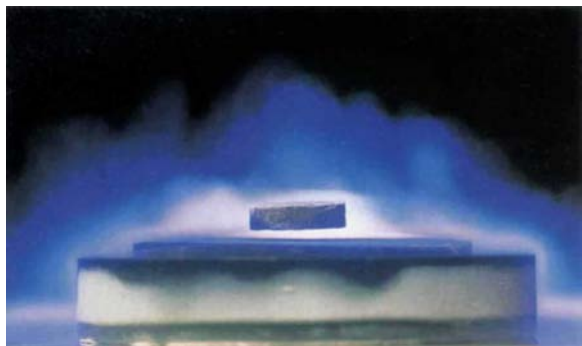
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Devices and developments

Paul M. Grant

Applied Superconductivity. Editor-in-chief Roger B. Poeppel. Elsevier. 12/yr. North, Central and South America \$649 (institutional), rest of the world £435 (institutional),

"Of making many books there is no end; and much study is a weariness of the flesh" (Ecclesiastes 12:12). In our present



Magnetic levitation by a superconducting ceramic.

age of information overload, this Old Testament adage (the Hebrew sages could have added wearisome to the spirit as well) echoes throughout almost every professional community whenever a new journal appears to compete for individual attention and library shelf space, both in increasingly short supply. Nonetheless, they keep coming, and in the opening months of 1993 the first issues of *Applied Superconductivity*, one more addition to the vast Pergamon stable (now itself owned by Elsevier), hit the newsstands of science. Is another journal really needed in a field whose workers struggle daily to dig themselves out from under the current avalanche of papers?

The answer is a definite maybe. Well, actually a no — those engaged in research into superconducting applications are going to continue to submit their most important results to well established and highly visible journals such as *Applied Physics Letters*, perhaps even the much-maligned yet ever-venerable *Physical Review Letters*. Among the newer journals, *IEEE Transactions on Applied Superconductivity* seems to have captured much of the readership targeted by Pergamon, especially those pursuing the development of electronic devices. Of seven major university and industrial institutions in the San Francisco Bay Area that run programmes in applied superconductivity, only two subscribe to *Applied Superconductivity*. All subscribe to *Applied Physics Letters* and *IEEE Transactions*.

To be fair, a very qualified yes may also be in order. There are so far no journals that focus solely on wire development and its application to large-scale power and magnetic devices. The paper appearing in the January 1994 issue of *Applied Super-*

conductivity by Y. S. Cha *et al.*, an excellent detailed analysis of critical current measurements relevant to wire 'embodiments', provides an example of the kind of submission that the editors need to encourage should they indeed wish to redirect their journal in such a direction.

One final small point: the cover of *Applied Superconductivity* displays the striking three-dimensional rendition of the crystal structure of La_2CuO_4 created by staff of the IBM Science Centre in Winchester, United Kingdom.

It would have been a nice touch to acknowledge this artistic contribution, if only in the introductory issue. □

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Rediscovery of unifying bonds

Fraser Stoddart

Supramolecular Chemistry. Editor J. L. Atwood and G. W. Gokel. Gordon and Breach. 4/vol. ECU1,359, \$1,632 (institutional); ECU282, \$339 (personal). Three volumes to be published in 1995.

EVERY so often the telephone rings and the news breaks that another journal is about to be launched. After some gentle preparation from an editor-in-waiting comes the inevitable question: "Will you serve on the editorial board?" If my wife answers the telephone, events usually unfold as follows. She comes right to the point: "Does he have to do anything?" And if the answer is "No", she says "Yes" and my name ultimately appears among some of the great and the good. If I answer the telephone, I ask: "Why another journal?" And because I rarely receive a very convincing answer, I beg for time to reflect on my decision. Delaying-tactics of this kind usually seal my fate and the editorial board eventually appears with many of my friends present and myself conspic-

uous by my absence. So it was my early experience with *Supramolecular Chemistry*.

With the experimental phase now behind it, I have to admit that here is a new journal for communications and papers — with reviews being "published only on an occasional basis" — that is fulfilling its mission as stated by the editors: that is, "to provide a timely presentation outlet for high quality work that bridges the traditional disciplines but which retains a chemical orientation". And sure enough, one finds good-quality publications across the spectrum of the well-known receptors (for example, cyclodextrins, crown ethers, calixarenes, cyclophanes, cryptands, spherands and so on) as well as highly original contributions to phenomenologically based (membranes, vesicles, sensors) and conceptually orientated (self-assembly, self-organization, self-replication and so on) research in the supramolecular domain.

The names of the leaders in the field are dotted throughout the early volumes and the presentation is of a uniformly high standard. Even colour turns up in occasional issues, although the plates are divorced from the publications they refer to and are relegated to the final entries just before the contents on the back cover of the journal. The overall style of the journal is user-friendly, with "Index Abstracts" featuring a graphical component. Supramolecular chemistry is a highly visual art form. Indeed, one can often predict the authorship of a publication from a glimpse of the pictures contained therein.

I also applaud the decision of the editors to feature one of the publications in each issue on the front cover. This practice, which should never be taken too seriously by established members of the community, does much to heighten everyone's interest in a journal. The encouragement that making the front cover brings to a young researcher has to be seen to be believed.

Above all, it is the part that this new journal is now playing in helping to reunite the once highly divided discipline of chemistry that is one of its main strengths. For some strange reason, perhaps scientifically based, the chemistry of the covalent bond has encouraged chemists to fragment into smaller and smaller groups with increasingly limited goals and horizons. At least at this early stage in its development, the chemistry of the noncovalent bond is encouraging chemists to come together and speak not only to each other but also to other scientists with greater authority. This is what supramolecular chemistry in its broadest sense is all about. □

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Gelling together

Richard Jones

Polymer Gels and Networks. Editor-in-chief T. Tanaka. Elsevier. 4/yr. £151, \$235.

Polymer Reaction Engineering. Editors A. Penlidis and K. F. O'Driscoll. Dekker. 3/yr. \$225 (institutional), \$112.50 (personal).

Trends in Polymer Science. Editor William Hawthorne. Elsevier. 12/yr. USA and Canada £490, elsewhere £318 (institutional); \$110, £69 (personal); \$55, £34 (student).

THE white of a fried egg and the epoxy resin matrix of a high-performance composite material may not seem to have much in common. But both are examples of gels, and the appearance of *Polymer Gels and Networks* is an expression of faith that such apparently disparate systems can be brought together within a single conceptual framework. The scope of the journal, then, is wide, ranging from the theoretical physics underlying the attempt to understand the gelation process, through polymer materials science, to biopolymer gels, important primarily as food materials but also because of their growing potential in biomedicine. In the near future lies the promise of gels as so-called intelligent or smart materials (although to my mind the term 'instinctive materials' reflects more accurately their capabilities) — materials that can react mechanically to changes in their environment, or membranes whose transport properties can be controlled electrochemically.

Despite its wide scope, the journal is at present modest in size — four issues a year, each with half a dozen papers or so. But the editors have been successful in attracting papers from a truly international community. In particular, the prominence of Japanese workers in the field of biopolymer gels is well reflected in the origin of papers published so far.

Polymer Reaction Engineering represents a much more closely defined community: those involved in the chemical engineering of polymerization, with a heavy emphasis on mathematical modelling and process simulation. From a superficial perspective, an outsider might take this to be a mature field. In fact, rapidly

increasing competitive pressures in the plastics industry have made the task of understanding and improving such existing processes an urgent and challenging matter. To some extent there are journals that already cover this ground, including *Polymer Engineering and Science* and *Journal of Applied Polymer Science*, but this latest publication may well be able to find a more specialized niche.

In contrast to this trend towards specialization, *Trends in Polymer Science* aims to bring unity to the whole field of polymer science, based on brief, invited, reviews of what the editors consider to be the exciting issues of the moment. It is an attractive formula, one that has clearly been most successful for the other *Trends* journals. But I feel that *TIPS*, as the publishers would no doubt like it to be known, has yet to find its feet.

First impressions are favourable; a colourful and well designed cover highlights the lead review article for each issue. But inside the journal has a dull and unattractive layout, only with very few poorly reproduced photographs. A "Viewpoint" column gives an invited author the chance to grind an axe of his or her choice; I welcome the attempt to stimulate debate and it is particularly pleasing that the authors of these articles are

industrialists as well as academics. But judging by the extreme paucity of letters published in response to some fairly provoking debating positions, an outsider might judge the polymer-science community to be not terribly lively, a conclusion that would be reinforced by reading the book reviews and conference reports, with their worthy and dully informative aura.

But it is the review articles that are at the heart of such a publication, and here I think there are also problems. Although several articles have been excellent, some have succumbed to a pitfall that faces all short reviews; they lack both the comprehensiveness of a full review article and the readability and immediacy of more popular science writing. The choice of topics, too, does not yet seem to have achieved a happy balance; underrepresented areas include polymer physics, particularly in its theoretical aspects, as well as the engineering side of polymer science.

I have no doubt, however, that the journal will succeed; it fulfils an important need, and its price will encourage personal subscriptions (the student rate is particularly good value). But it still has some way to go before it lives up to its potential. □

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Competing chemical companions

David C. Rees

Bioorganic and Medicinal Chemistry. Editor-in-chief C.-H. Wong. Elsevier. 12/yr. North, Central and South America \$700, elsewhere £455.

DURING the 1990s, several new players have entered the primary literature of medicinal chemistry and bioorganic chemistry (for example, *Bioorganic and Medicinal Chemistry Letters*, *Bioorganic and Medicinal Chemistry*, *Current Medicinal Chemistry* and *Medicinal Chemistry Research*). Some of these have become established as essential reading for scientists wishing to keep abreast of the latest results in these areas (see *Nature* 359, 458; 1992).

Assessing *Bioorganic and Medicinal Chemistry* in its second year of publication, one is tempted to look back at the effects of this flourishing development of new journals. They reflect both the emergence of medicinal chemistry as a major discipline in its own right and the growing scientific and commercial interest in understanding how chemical structures control biological processes at a molecular level. The new journals also provide an outlet that will motivate medicinal chemists to present their results at a time when the traditional distinctions between organic chemistry, biology and molecular

pharmacology are becoming increasingly blurred.

As its name suggests, the new journal is closely related to *Bioorganic and Medicinal Chemistry Letters*. Readers of the other Pergamon duet, *Tetrahedron* and *Tetrahedron Letters*, will be familiar with the concept of a rapid publication journal for short preliminary communications (the *Letters*) with more detailed accounts, including full experimental procedures, appearing in *Bioorganic and Medicinal Chemistry*. Two desirable hallmarks of the Pergamon journals are retained: the graphical abstracts, which allow readers to scan the contents rapidly, and occasional "Symposia-in-Print", the first of which appeared in the June 1994 issue and attracted 24 papers on biological catalysis.

If this new journal is to be regarded as a success it will, like any other, have to compete for readers and budgets. It is clearly different from the established organic chemistry literature because, in addition to synthesis, it requires target biological data (on, for example, enzyme activity or receptor binding). A more appropriate comparison, however, is with the American Chemical Society's prototype *Journal of Medicinal Chemistry*, and here the added value is less clear. The scope of both publications is similar (all areas of medicinal and bioorganic chemistry) but

Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1994. This information may not be complete in all cases. Readers interested in a particular journal are therefore advised to check prices with the publisher before subscribing.

so far *Bioorganic and Medicinal Chemistry* has significantly fewer papers and is published less frequently (monthly as opposed to twice monthly), although the costs of both journals are similar.

In summary, *Bioorganic and Medicinal Chemistry* has not yet displaced the *Journal of Medicinal Chemistry* as the gold standard, but it is certainly worthy of serious consideration as an addition to any library subscribing to the latter. □

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In fine mettle

Carl C. Koch

Intermetallics. Editors, R. W. Cahn, C. T. Liu, G. Sauthoff, M. Yamaguchi. *Elsevier*. 4/yr. £219, \$335.

INTERMETALLICS — ordered compounds composed of two or more metals — have generated interest in both scientific and engineering circles for many years owing to their often unique properties. More than a decade ago, several 'break-throughs' were reported in the ability to make these normally very brittle materials ductile. This led to a great resurgence in research on intermetallics as structural materials with possible applications at elevated temperatures. Today, intermetallic compounds are probably the most studied class of metallic materials. Research on intermetallics is published throughout the range of journals on materials science and condensed-matter physics as well as in large symposia proceedings.

At four issues a year, *Intermetallics* can hope to publish only a small proportion of this extensive literature. But the editors are in the enviable position of being able to limit their journal to papers of the highest quality. Review articles, research articles, short communications and comments on papers are all solicited.

The production quality is also excellent. This is important for materials science journals, where clear and accurate photographs (such as transmission electron micrographs) are often critical to the interpretation of experimental results.

The journal should be attractive for authors: there are no page charges and 25 reprints are supplied free. The subscription price unfortunately puts the journal out of reach of most individual subscribers, and given the tight budgets of libraries, it may also prevent many institutions from access to what is an important new journal in a vital field. □

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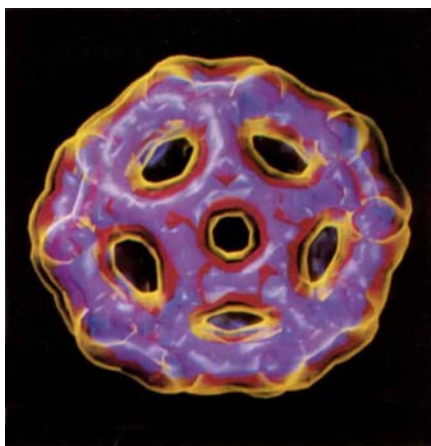
Fullerene fever

Arthur F. Hebard

Fullerene Science and Technology. Editor T. Braun. *Dekker*. 4/yr. \$250 (institutional); \$125 (personal).

IN the foreword to the first issue of this new journal, the editor opens with the statement that "some facts indicate that fullerene fever is more than just a fashion". He then points out that the general fascination with these beautiful molecules and well established but broadly dispersed research activity justify the need for a dedicated journal that will "provide an interdisciplinary and cross-disciplinary platform for international communication".

One might justifiably ask, however, whether the cure for fullerene fever is the birth of a new journal. It is not clear whether fullerene science will develop a tradition of its own or become assimilated into the more general field of carbon science. For this new journal to be successful, it will therefore have to create a unique niche and cater for a multidisciplinary readership by attracting seminal contributions from a broad spectrum of researchers. This is a daunting task



Electron-distribution simulation of fullerene.

because many potential contributors will be tempted to submit to the numerous established journals that already have a wide readership and which often have sections or special issues dedicated to fullerenes.

Fullerene Science and Technology does have a highly qualified multinational editorial board, which should help in the soliciting of papers. Five of the editors have shown their commitment by writing or co-writing articles in the inaugural issue. Two of the four volumes reviewed are proceedings issues devoted to the Fourth and Fifth General Symposia on Fullerene Science and Technology held in Tokyo, Japan. Unfortunately, the reduction by more than half in the number of

contributions to successive symposium issues may indicate lagging interest — a portent of trouble for a struggling new journal.

The scope of this journal at its incubation stage is determined by the papers submitted. Although in general the quality of work reported is fairly high, a few of the manuscripts seem rather preliminary and inconsequential. A more varied format that included brief communications, comments, guest editorials, and technical notes might increase the appeal of the journal. □

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Looking good

Ian A. Walmsley

Pure and Applied Optics. Editor M. Bertolotti. *Institute of Physics/American Institute of Physics*. 6/yr. USA, Canada and Mexico \$272, elsewhere £132.

THIS journal, published on behalf of the European Optical Society as Part A of a two-part set, covers just about all of classical optics: that is, those optical phenomena that do not require a description in terms of quantum fields. So nonlinear optics, laser physics and spectroscopy are included. In fact, topics under the heading of quantum optics are specifically excluded, presumably coming under the jurisdiction of Part B.

Although the reasoning behind the separation is clear (and notably different from that behind the splitting of the *Journal of the Optical Society of America* in 1984), it sometimes leads to a strange juxtaposition of articles; one on caustics in ray propagation, for example, sandwiched between papers on phase conjugation and mode-locked laser theory.

The editorial board is large, and its members are all eminent scientists and engineers, giving the journal a fairly international perspective and placing it firmly in the ranks of other distinguished titles. Both full-length papers and letters (of specified maximum length) are published, and there are also book reviews and lengthier review articles.

An attractive feature for authors is the absence of page charges (and this even with a fairly modest library subscription rate), and, as a further nice touch, a few off-prints are provided gratis by the publisher. The journal will be an important addition to any comprehensive optics research library. □

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Fending for themselves

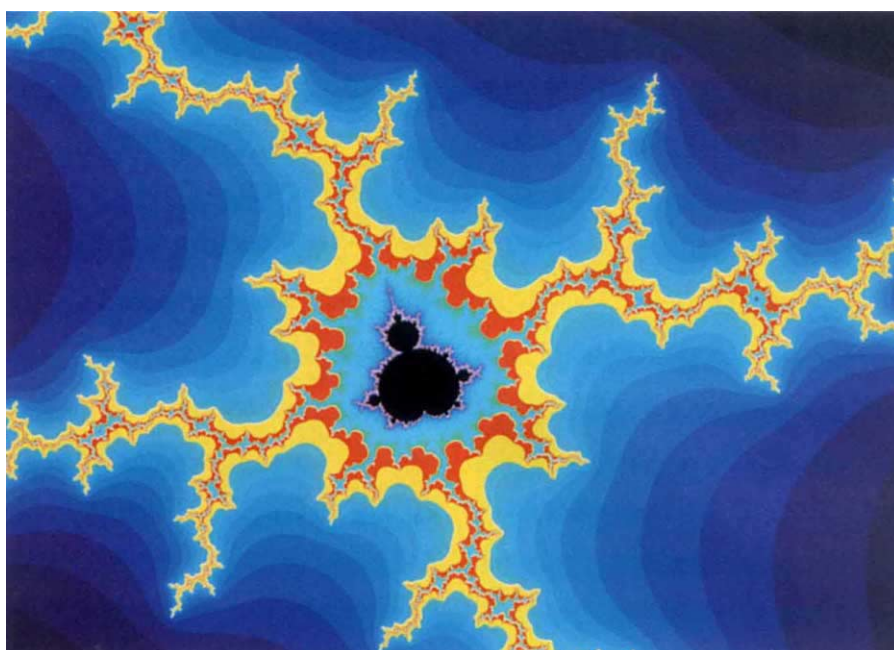
Ian Stewart

Fractals: An Interdisciplinary Journal on the Complex Geometry of Nature. Honorary editor B. B. Mandelbrot; managing editors M. Matsushita, M. Shlesinger and T. Vicsek. *World Scientific*. 4/yr. \$240 (institutional); \$96 (personal/institutions in developing countries).

OVER the past decade the subject of fractals has matured into a distinct area of scientific enquiry. Like all active research areas, its boundaries are fuzzy and its sub-

ject matter is not always precisely defined. Mathematically, fractals are 'recursive geometry' — geometric forms with structure on all scales; physically they are natural phenomena with structure on an unusually wide range of scales. The mathematics is a model of reality, not a faithful representation — as usual.

with occasional colour. Scientifically it is fairly impressive too, with an attractive mix of theory and experiment, simulations and proofs. The first two volumes already cover most of the main areas in which fractal geometry has become an accepted tool: fracture surfaces, aggregation processes, image compression, $1/f$ noise. They also include papers in many new areas. A good example, by Eshel Ben-Jacob and collaborators, discusses the complex patterns that form during the growth of colonies of bacteria. These structures range from tree-like branching forms to dendritic clusters to compact discs, and they are studied experimentally and by computer simulation. Somewhere towards the opposite end of the scientific spectrum are papers such as that by Lajos



Fractal geometry — a computer graphics image entitled "A Birth of Lightning", derived from the Mandelbrot set.

ject matter is not always precisely defined. Mathematically, fractals are 'recursive geometry' — geometric forms with structure on all scales; physically they are natural phenomena with structure on an unusually wide range of scales. The mathematics is a model of reality, not a faithful representation — as usual.

As early as 1984, various publishers conceived the idea of a specialist journal devoted to fractals. Their creator, Benoît Mandelbrot, sensibly declined all invitations to edit such a journal on the grounds that it would be premature. The way to publish an interdisciplinary subject is to disperse it among all the journals of all the appropriate disciplines. Yet now we have a specialist journal. What has changed? Fractals have grown up, and it is time for the fledgling science to leave the nest and fend for itself.

Fractals is physically impressive: large format, glossy pages, profusely illustrated

Nyikos and collaborators, who obtain numerical calculations of various fractal dimensions (box dimension, information dimension, mass exponent) of drawings by artists such as Picasso, Dürer, Rembrandt and Munch.

Several volumes are special issues of conference proceedings. For example, volume 2 number 3 is the proceedings of the "Fractals In Engineering" conference, held in 1994, which concentrates mainly on the use of fractal methods in image processing.

Fractals demonstrates the breadth, depth and vitality of the area, and it is essential reading for anybody whose research involves structures and forms that are irregular yet have a definite character of their own. □

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Top secrets revealed

Jon Keating

Russian Journal of Mathematical Physics. Editor-in-chief V. P. Maslov. Wiley. 4/yr. USA \$340, Canada and Mexico \$380, elsewhere \$395.

IT used to be with some trepidation that one opened a Russian journal devoted to mathematical physics: although researchers from the former Soviet Union have undoubtedly led the world in this field for several decades, their papers have, unfortunately, often been unworthy of the work they were meant to describe. In all too many cases, important results have lain hidden within incomprehensible notes and articles.

In the past, the main motivation of many Russian authors seems merely to have been to stake their claim to a theorem, rather than to explain the ideas and methods underlying its proof. Articles were often excessively brief, with key steps and arguments sometimes entirely omitted. Also, the writing, translation and typesetting were generally rather poor, with frequent mistakes and typographical errors.

So the *Russian Journal of Mathematical Physics* is a welcome step in developing a uniform quality in the literature. The papers are of a high standard; in particular, they are generally extremely well written, carefully translated and clearly typeset (in the usual Western style). There has obviously been considerable effort to make them readable and easily understandable to nonspecialist mathematical and theoretical physicists. The intended audience is essentially the same as that catered for by the *Journal of Mathematical Physics*, which the new journal should complement successfully.

"Due to the cardinal changes in world politics and the conversion of the military industry we will be able to publish papers that previously would have been contained in secret reports", claim the editors in the journal's stated aims and scope. I, for one, will certainly be curious to see the sort of results generated in this area by the remarkable levels of funding lavished on defence-related research over the past few decades. □

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Autumn Books

Nature's next review supplement is Autumn Books, which will appear in the issue of 17 November 1994.

Computer programs running wild

Antonia J. Jones

Evolutionary Computation. Editor-in-chief Kenneth De Jong. MIT Press. 4/yr. USA \$125, Canada \$148.73, elsewhere \$139 (institutional); USA \$45, Canada \$63.13, elsewhere \$59 (personal); USA \$30, Canada \$47.08, elsewhere \$44 (student/retired).

THERE has long been a pressing need for an eclectic journal dealing with 'genetic algorithms', the phrase coined by the US school stemming from the work of John Holland, 'evolutionary programming', originally developed by L. J. Fogel, A. J. Owens and M. J. Walsh, again in the United States, and 'Evolutionstrategie', as studied in Germany at around the same time by I. Rechenberg and H.-P. Schwefel. Much of this early work took place in the mid-1960s, although the European and US schools seemed largely unaware of each other's existence for quite a while.

The First International Conference on Genetic Algorithms (ICGA) was not in fact held until 1985. It was there that the idea of a journal was initially discussed, but it has taken a further nine years to turn it into a reality. Meanwhile, there were a limited number of options for publishing papers in what had become a rapidly expanding field. The ICGA meetings and later the European Parallel Problem Solving from Nature Conference were the main outlets for work in the field, but conference papers are necessarily rather compressed. And since 1989, the Santa Fe Institute series of annual volumes, edited by C. G. Langton *et al.* (Addison-Wesley), have provided a new perspective and the opportunity to publish longer papers.

Evolutionary Computation is therefore a much needed and welcome addition to the field and its title is appropriate for the proposed coverage, which aims to include mathematical and biological foundations of evolutionary computation, characterization of appropriate problems, parallel models and implementation issues of evolutionary algorithms, evolutionary approaches to machine learning and artificial life, the evolution of neural networks, emergent properties (a rather suspect phrase, I have often thought) and applications of evolutionary computation to problems in science, engineering, economics and so on. Because J. Koza is on the editorial board, it is a safe bet that genetic programming will also be covered.

The first volume begins with "An Overview of Evolutionary Algorithms for Parameter Optimization" (T. Bäck and Schwefel), which, despite the rather dry title, makes fascinating reading and contains an excellent, compact introductory guide to the literature. Subsequent issues contain a variety of interesting papers, all of high standard. Setting aside the inevitable occasional teething problem, the production team have put together a

first-class product.

The politics of a developing field are often fraught with difficulty as alternative viewpoints contend for recognition, and Kenneth De Jong is to be congratulated on making a success of a challenging role. □

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Bringing home the bacon

David Willshaw

Neural Computing and Applications. Editors David Bounds, Howard James and Rodney Goodman. Springer. 4/yr. £130 (institutional); £33 (personal).

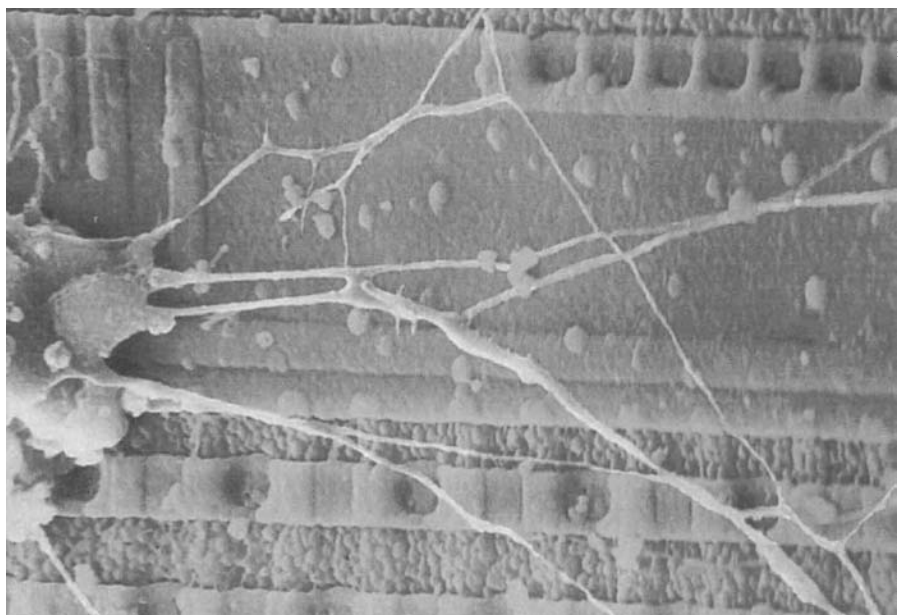
THE field of neural networks (known variously as neural computing, parallel distributed processing or connectionism) is concerned with the development and use of novel algorithms intended to mimic (to a greater or a lesser extent) the workings of the brain. Many new journals covering this interdisciplinary research area have appeared over the past five years. But very few have been targeted at applications — surprisingly few given that many neural-net practitioners fervently believe that

the fruits of their research are highly applicable.

This UK-based journal aims to publish "original research and other information in the field of practical applications of neural computing". It also serves as the house journal for the Neural Computing Applications Forum, which holds discussion meetings in the United Kingdom and Europe on topical applications of neural networks. In the issues I received, about a third of the papers would not have been out of place in a middle-of-the-range neural-network journal. The rest describe applications in a variety of areas — for example, forecasting share prices, models of control systems for chemical plants, medical diagnosis and, intriguingly, pig-carcass grading.

In my experience, people working on commercial applications are good at describing what they have achieved, but are rather more coy about how they did it. It is encouraging that in this journal, technical descriptions are reasonably full. In most cases, it is shown that an algorithm based on a neural network (mainly of the back-propagation or self-organizing type) can solve the problem and that neural network variant A is better than neural network variant B. More information would have been welcome on how the neural-network algorithms compared with more standard methods. Perhaps the journal could make a contribution by instituting a set of benchmarks? If it can concentrate on publishing well-validated examples of applications in the field of neural networks then it will perform a useful service. □

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Biohybrid — human nerve cells growing on a silicon chip.

Going with the flow

Michael A. Leschziner

International Journal of Computational Fluid Dynamics. Editor-in-chief W. G. Habashi. Gordon and Breach. 4/vol. ECU774, \$930 (institutional); ECU180, \$196 (personal). Two volumes in 1995.

COMPUTATIONAL fluid dynamics is one of several disciplines that have responded to developments in high-performance computers in much the same way as Californian and Texan boom towns reacted, around the turn of the century, to the discovery of gold and oil underfoot: a rapid, uncontrolled growth with very flaky edges, punctuated by tentacles sent out in all directions without a firm core infrastructure to support outlying areas. Matters have not been helped by the fact that the field, although a theoretically challenging branch of nonlinear mathematics and physics, is of outstanding practical importance in thermofluids engineering and has therefore been the victim of irresponsible exploitation. A further adverse consequence of the relevance of computational fluid dynamics to numerous industrial applications has been a proliferation of application-specific and often weakly founded variants of what are, essentially, sets of generic numerical tools that constitute the discipline. Recently, however, there has been an increasing realization that the key issues of computational fluid dynamics — among them numerical accuracy, resolution, computational economy and realism of the mathematical closures approximating physical processes — must be addressed or revisited in a systematic, focused and critical fashion.

This new journal wishes to be seen as distinct from others in being a specialized forum "serving the discipline as a unit and gathering under one title [its] main aspects". It declares its principal aims to include: "timely dissemination of innovative research ideas"; putting "emphasis on papers dealing with efficient methods to produce accurate predictive numerical tools for flow analysis"; being a "truly interdisciplinary forum" for computational fluid dynamics by publishing "latest advances in numerical methods in fluid dynamics for the aeronautics, astrophysics, environmental and process fields". These are all laudable objectives suggesting a firm intention to promote careful research on generic issues.

The first four issues do not suggest to the critical reader expecting a fresh approach that the journal is on its way to becoming a distinctive forum of the kind to which it aspires. The mix of articles does not distinguish itself dramatically from that in several other periodicals devoted to the field, and there does not seem to be a clear theme or a new philos-

ophy behind the selection of contributions. Indeed, the reader is occasionally confronted by some startling subject sequences, moving, for example, from supersonic flow by way of the analysis of data on surface-water quality to turbulence modelling. Although several articles are strong and present impressive achievements, especially in finite element methods, some are mediocre, and a few are rather weak. In at least two instances, complex flows are analysed with first-order convection schemes, which gives no credit to the papers or the journal. Few of the papers include statements on numerical accuracy, grid-independence and error bounds — the inclusion of these is a must for any journal laying claim to rigour. On a more mundane level, the linguistic quality of some papers is not high, and the quality of graphical material is variable. Also, there appears to be no uniformity in

the nomenclature, the inclusion or exclusion of keywords or even in the use of the heading "Abstract" or "Summary" at the beginning of articles. A pleasing feature of the journal, however, is that it permits "Short Communications", thus promoting rapid dissemination of new developments.

In summary, unless it gains a more distinct profile, the journal will establish itself as only one of several alternative outlets for research on computational fluid dynamics. At present, there continues to be a gap in the 'CFD market' for a specialist journal with a fresh approach that places emphasis on rigour and on carefully verified improvements to the generic constituents of the discipline. □

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Listening while learning

Sanjiva K. Lele

Journal of Computational Acoustics. Editors-in-chief Ding Lee and Allen D. Pierce. World Scientific. 4/yr. \$190 (institutions); \$95 (personal).

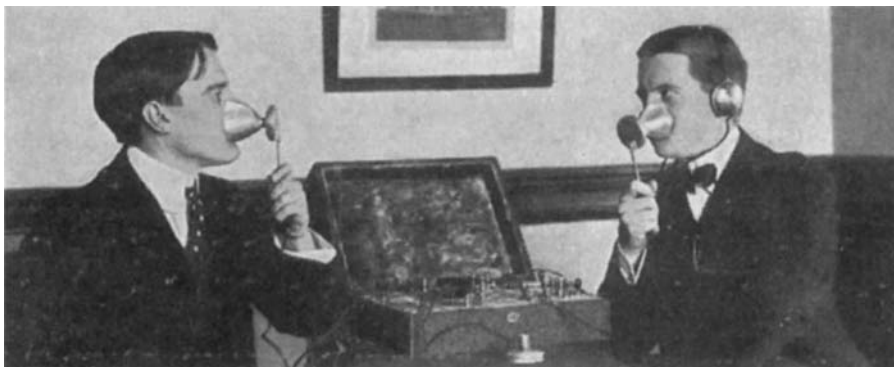
ACCORDING to the editors of *Journal of Computational Acoustics*, the traditional compartmentalization of acoustics and related branches of science and engineering has not been conducive to the exchange of ideas and information. This new journal intends to provide scientists and engineers — whether their main interest is in oceanography, aerodynamics, applied mathematics, noise control, meteorology, geophysics, bioengineering, nondestructive testing, mechanical systems or computer science — with a forum for sharing their achievements in, and insights into, the broad field of computational acoustics. The authorship of early issues reflects these diverse communities.

But why limit the potential of this cross-fertilization of ideas? Why restrict coverage to computational acoustics?

There is perhaps a feeling that recent developments in computer hardware and numerical algorithms will allow a new computational assault on a wide range of problems in acoustics. Computational tools for acoustical analysis and design with respect to structural engineering are also on the horizon. And there is increasing concern about noise pollution, leading to a push for new technologies that are quieter or capable of controlling noise.

Computational methods may well help to develop and perfect these technologies, but their precise role is difficult to predict. Some areas may require an engineer's blend of theory, experiment and computation. The journal does have a niche, but its effectiveness will depend on whether it can encourage a proper dialogue among the applied acoustics communities it aims to embrace. □

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Mary Evans

The "Acousticon", an instrument enabling the deaf to hear, was invented by M. R. Hutchinson in 1903. He used it successfully on thousands of deaf mutes in New York.

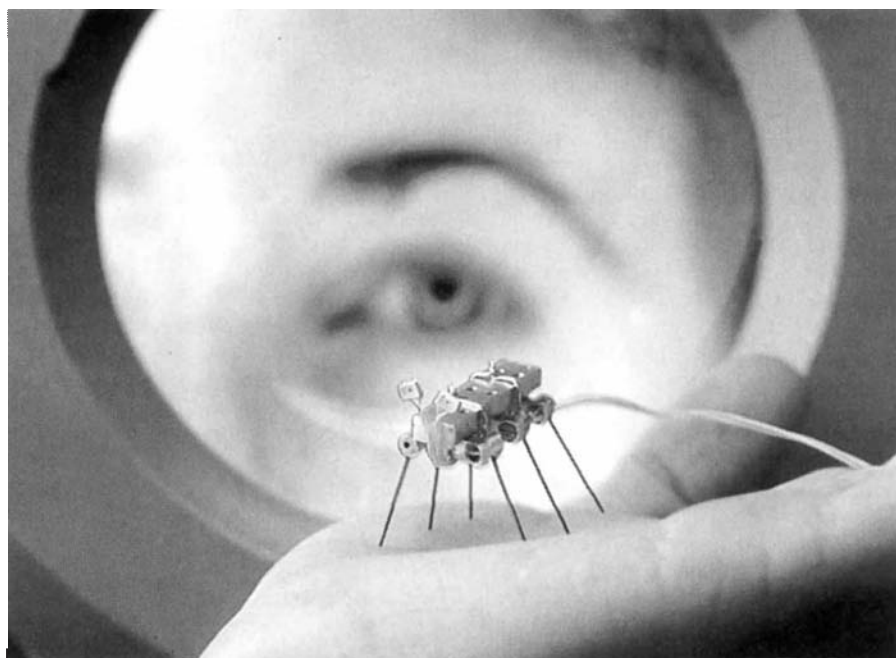
From animals to animats

Dave Cliff

Adaptive Behavior. Editor Jean-Arcady Meyer. MIT Press. 4/yr. USA \$115, Canada \$138.03, elsewhere \$129 (institutional); USA \$50, Canada \$68.48, elsewhere \$64 (personal); USA \$35, Canada \$52.43, elsewhere \$49 (student/retired).

THE scope of this journal takes some explaining. Contributions originate from a wide range of disciplines, including artificial intelligence, behavioural ecology, ethology, neuroscience, psychology and robotics. The journal's unifying theme is the study of issues in the analysis or design of autonomous agents: that is, agents

ing article, and the last issue includes indexes to the volume. Although future volumes might see the inclusion of short papers, book reviews and so on, the decision to place early emphasis on substantial and important papers has helped to establish the journal as the authoritative publication in the field. Papers range from neurobiology to robotics, but the constant theme of understanding adaptive behaviour in both natural and artificial systems lends coherence and accessibility to research projects that might otherwise seem superficially disparate. Most of the papers published so far have been of good quality and are well written for the



MIT-bred robot 'gnat', complete with photodetectors and logic processors.

capable of coordinating perception and action for extended periods of time in unknown and potentially hostile environments. Such coordinated activity is referred to as adaptive behaviour if it increases the agent's likelihood of survival.

The autonomous agents studied may be biological (such as animals) or artificial ("animats": creature-like mobile robots, or software agents inhabiting virtual realities). Increasingly, analysis of biological mechanisms underlying the generation of adaptive behaviours is aided by the use of computer simulations or robot models; similarly, designers of animats often draw inspiration from findings in biology. This journal offers a unique forum for the exchange of ideas and the study of adaptive behaviour in general.

Each issue in volume one contains four long articles (around 15,000 words); the first issue also contains an inaugural lead-

journal's necessarily multidisciplinary audience. The production standards are high, with attractive typographical layout on acid-free paper in a compact (roughly A5) format.

The editor, eight associate editors and 26 editorial board members account for most of the influential researchers in this young field (the first international conference on simulation of adaptive behaviour was held in 1990). It is difficult to conceive of new rival journals dislodging *Adaptive Behavior* from its niche; its survival prospects would seem to be good. The subscription rates are very reasonable; for scientists with interests in the common ground where studies of natural and synthetic autonomous agents meet, it should become essential reading. □

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Bird imbalance

Lorraine Maltby

Journal of Aquatic Ecosystem Health. Editor-in-chief M. Munawar. Kluwer. 4/yr. DFL356, \$222.50.

Ecotoxicology. Editors D. B. Peakall and L. Shugart. Chapman and Hall. 4/vol. USA and Canada \$190, Europe £110, elsewhere £120 (institutional); USA and Canada \$79, Europe £45, elsewhere £45 (personal).

GIVEN the growing concern over the impact of humans on the environment, it is not surprising that publishers should try to tap into this interest by launching new ecotoxicological journals. These two are the most recent contenders. They have similar aims and scope in that they publish original research papers and review articles on the effects of toxic chemicals on populations, communities and ecosystems. Neither journal is restricted to specific taxonomic groups although *Journal of Aquatic Ecosystem Health* is limited to aquatic systems.

Despite similar aims, the types of articles published in the first two volumes are quite different. Whereas the majority in *Ecotoxicology* are original research papers, most in *JAEH* are reviews. This emphasis on review-type articles reflects the fact that seven of the first eight issues of *JAEH* contain papers resulting from three scientific meetings. If *JAEH* is to become more than a vehicle for conference proceedings it must begin to attract more unsolicited papers describing original research.

Both journals have a North American bias in authorship, although this is much greater for *JAEH* (72 per cent compared with 44 per cent). There is also a bias in subject matter. Roughly 40 per cent of the papers in *Ecotoxicology* are concerned with the effects on birds of chemicals, principally pesticides. This imbalance needs to be addressed if the journal is to fulfil its intention of favouring no particular taxon or biome.

Ecotoxicology is an expanding area and there has recently been a shift from studies describing the effects of a single chemical on individual organisms to studies concerned more with understanding and predicting the effects of chemicals on populations and communities. There is therefore a need for journals that publish the results of such studies. Several established journals already do this, and unless *Ecotoxicology* and *JAEH* can establish unique identities they will have difficulty competing.

There is a potential niche for publications that adopt a holistic approach to ecotoxicology, as pointed out by John Cairns Jr in articles in the inaugural issues of these two new journals. Given their similarity in scope, however, it is unlikely

that both will become mainstream. *JAEH* states explicitly that it aims to publish research with a holistic perspective, so it ought to be in the best position to exploit this 'holistic market'. But, on the basis of the number of unsolicited papers published, it seems that *Ecotoxicology* is attracting the most interest. □

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Cooperate, diverge or die?

Colin B. Munn

Journal of Marine Biotechnology. Editors J. T. Baker, H. O. Halvorsen, Y. LeGal, Y. Okami and S. Miyachi. Springer. 4/yr. \$108 (institutional); \$80 (personal).

WHAT exactly is biotechnology? Is it the application of molecular 'biotechnological' methods to investigate basic questions in biology or is it specifically concerned with industrial applications that exploit biological resources? I recall all sorts of semantic discussions during the emergence of the 'new' biotechnology in the late 1970s and 1980s, catalysed by the developments in recombinant DNA technology. The question becomes important when one asks whether there really is a discipline of 'marine biotechnology', and whether research should be focused under this heading or under the specific sub disciplines, such as microbiology, algology, fish biology, invertebrate studies and the many other areas relevant to the

marine biosphere.

The term 'marine biotechnology' probably first came into use in the mid-1980s, and in 1989 the first international symposium on the topic was held in Japan. This successful venture now seems to be established as a regular triennial event, with subsequent meetings in the United States and Norway. There is clearly a body of scientists who share a common interest in this exciting topic, which holds much promise for an increased basic understanding of the marine environment and for the potential use of resources. This is the rationale behind the launch of this journal. The stated aims are to provide a central information source and forum for scientists working in this field, and to focus on the industrial use of marine bioresources.

The journal is good value and well produced, with an attractive clear layout. Most of the original papers are clear and concise (3–6 printed pages). There are also excellent short mini-reviews, which are particularly useful for promoting interdisciplinary awareness. 'Rapid Communications', a section for publication of new results of special interest, is especially welcome. The date of receipt of manuscripts is not recorded, so it is impossible to judge the normal publication time for papers. There also seems to be the opportunity for editorial comments and correspondence. The editorial board consists of distinguished experts across a wide range of relevant disciplines.

An obvious comparison must be made with the other new journal in this field, *Molecular Marine Biology and Biotechnology*, reviewed in last year's New Journals supplement (*Nature* 571, 576; 1993). There is a definite need for a single forum for gathering together work related to this growing field. If both journals are to

thrive, I suspect that they will need to develop rather different philosophies and approaches. *Journal of Marine Biotechnology* would be best focused on applications — the industrial use of marine organisms and environmental processes and remediation — whereas *Marine Molecular Biology and Biotechnology* should concentrate on more basic studies that use molecular methods to investigate topics such as biodiversity and marine ecology. It would be even better if the publishers were to collaborate to produce a single comprehensive journal — that really would be useful! It is encouraging that they have already agreed to cooperate in the publication of the proceedings of the 1994 International Marine Biotechnology Conference, held last month in Tromsø, Norway. □

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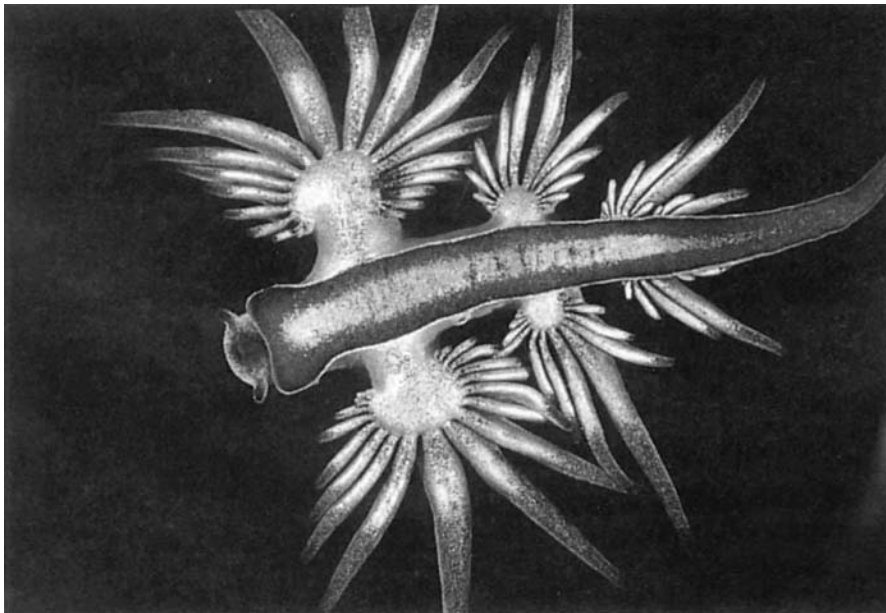
Scarcity or abundance?

Michael B. Usher

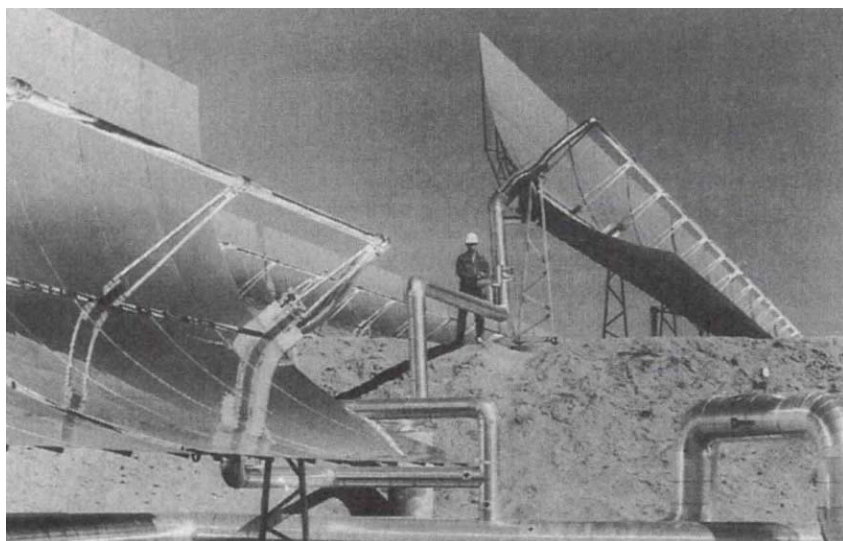
Biodiversity and Conservation. Editor-in-chief Alan T. Bull and Ian R. Swingland. Chapman and Hall. 6/yr. Europe £190, USA and Canada \$325, elsewhere £200 (institutional); Europe £55, USA and Canada \$99, elsewhere £55 (personal). **Biology and Environment: Proceedings of the Royal Irish Academy.** Editors Martin Steer and Paul Giller. Royal Irish Academy, Dublin. 3/yr. IR£90 (institutional); IR£25 (personal).

MANY journals have seen the need for snappier titles — long-established journals have shortened their names, and newly published journals have chosen short titles. This trend is seen with these two journals, one new and one old with a new name.

Biology and Environment was launched in March 1993 as "an honourable successor to the original Section B". It aims to have a wide remit spanning "the internal structure and function of living systems on the one hand and investigations of the ecosystem and biosphere levels on the other". The papers appear to have two particular foci: ecological and ecotoxicological studies. These are not, however, the only subjects covered: there are papers on biochemistry of fish muscle, a database for peatlands, parasites of two trout species, and palaeoecology in western Donegal. Despite this disparity of topics there is a common theme; the Irishness of all of the studies. I previously had no idea about the amount of research in Ireland on the otter (*Lutra lutra*), a species that is the subject of eight of the 33 papers



Nudibranch or naked sea slug *Glaucus atlanticus*, a marine gastropod mollusc found on the sea bed, principally in the Atlantic.



Concentrating sunlight — between 1984 and 1990, Luz International Ltd designed and built nine solar thermal plants at three sites in California's Mojave Desert. The plants, which use parabolic mirror troughs, are the largest solar energy projects in the world. Taken from R. Golub and E. Brus's *The Almanac of Renewable Energy: The Complete Guide to Emerging Energy Technologies* (Holt, \$19.95 (pbk)).

in the first four parts.

With many nations developing biodiversity plans following the UN Conference on Environment and Development held in Rio de Janeiro in 1992, the title *Biodiversity and Conservation* for a new journal must be right. First published in 1992, the editorial policy is to include both contributed papers and special issues on individual topics. For example, one part in volume 2 contains a collection of seven papers on the theme "peatlands and people". These papers provide an overview of peatland archaeology and palaeoecology, conservation and the use of peatlands industrially and for forestry, but, apart from one paper on botanical diversity, it is difficult to gain an understanding of the biodiversity of peatlands. A similar criticism could be made of other parts of volumes 2 and 3 where "conservation" is the predominant theme and "biodiversity" seems lost in the ecological and conservation studies. Certainly the genetical aspects of biological diversity are poorly represented in the papers, but it is useful to see papers on zoos, cryopreservation of seeds and the economics of nature tourism.

Both journals contain collections of papers and a selection of book reviews. The number of reviews in *Biology and the Environment* is small, but the review section is clearly lively in *Biodiversity and Conservation*. Both journals have a few short articles in some of their parts, but these sections of comment, recent discoveries and so on are certainly far from vibrant. With the excitement of conservation, the environment and the interactions of humans with nature, there is still scope for the development of fora that recognize these critical issues as we approach the twenty-first century. □

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O'er the land of the free

Jeff Wilson

Land Contamination and Reclamation.

Editor D. Davis. EPP, 52 Kings Road, Richmond, Surrey TW10 6EP, UK. 4/yr. United Kingdom £69, elsewhere £79 (institutional); United Kingdom £35, elsewhere £45 (personal).

PUBLIC concern about environmental matters shows no sign of slackening. Rather the reverse: there is now much less willingness to tolerate pollution, or the threat of pollution, even when its damaging effects are reckoned to be barely perceptible. Sharp disputes between fishing and forestry interests over the possible consequences of acid deposition on forested catchments in relatively pristine areas of Scotland illustrate this heightened awareness, particularly when it involves problems of contamination of soils.

The state of the environment is of over-arching interest to all sectors of society, both urban and rural, impinging as it does on many aspects of community, commercial and political life. This new journal is a successful attempt to provide a wide audience with informed comment on, and in-depth analysis of, all aspects of land contamination and remediation. It is definitely not only for specialist environmental scientists; although it contains well researched and extensively referenced articles, there is also an attractive mix of other contributions that will immediately appeal to outsiders. For example, early issues feature authoritative papers on land contamination by chlorinated dioxins and furans, statistical sampling design for contaminated sites, human health risks from ingestion of contaminated soil and the effects of contaminated land on ground water.

There are also several papers, mainly

from industrial sources, that have a practical slant and refer extensively to reports by consultancies and agencies. As one might expect, commercial considerations are very much to the fore. Topics covered include stabilization of contaminated soil, soil washing and quality-assurance principles in the survey and analysis of contaminated land. These papers are marked by a complete absence of references to the scientific literature, yet despite — or because — of this they are nonetheless extremely useful from an applied point of view.

A further feature of the journal is the inclusion of notes on current land-remediation projects; news of new equipment, services, courses and conferences; book reviews; clean-up policies in different countries; and reports on the SITE programme of the US Environmental Protection Agency. There is something for everyone in this journal, and it is reasonably priced and well produced. The editor and his advisory board have struck a commendable balance. The stated aim of the journal is "to supplement original research and discussion papers on environmental, technical and public-health issues with full coverage of the commercial and legal context" and in this they have been eminently successful. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1994. This information may not be complete in all cases. Readers are therefore advised to check prices with the publisher.

Greening of politics and management

Michael Jacobs

Environmental Politics. Editors Stephen Young and Michael Waller. *Frank Cass*. 4/yr. £98, \$140 (institutional); £42, \$55 (personal).

Greener Management International. General editor Grant Ledgerwood. *Interleaf, Exchange Works, Sidney St, Sheffield S1 3QF, UK*. 4/yr. £150, \$250 (institutional); £50, \$80 (student/developing countries).

EVERYONE now recognizes the importance of 'environmental issues' in contemporary politics. But what are the political processes by which such issues get defined and placed on the policy agenda? What exactly is the 'environmental movement'? Are green parties the harbingers of far-reaching change, or merely temporary curiosities? Do green ideas add up to a coherent political theory?

These are the questions with which *Environmental Politics* is concerned. Its particular specialism is the study of the environmental movement and green parties in industrialized countries: in just two years it has become the principal forum for scholarly debate in this field.

In the analysis of policy, *Environmental Politics* has more competition from other

journals, but its focus on the political dynamics of change — on issues such as Antarctica, protection of whales and the development of environmental regulation in the United Kingdom — provides a valuable complement to the more technical orientation found elsewhere.

Perhaps surprisingly, the journal has not yet sustained a vigorous debate in the field of green political theory. Another criticism might be the exclusive focus on the industrialized countries. Many of the most interesting and important developments in environmental politics are now taking place in the South; and, as the 1992 'Earth Summit' in Rio de Janeiro showed, it is vital that Northern commentators and politicians understand the perspective of developing countries.

Unusually for an academic journal, *Environmental Politics* includes short, newsy papers (up to 2,000 words) covering recent political events and developments. Subjects have included the fortunes of green parties in elections in Germany, France, Italy, the United Kingdom, Australia and elsewhere; the activities of green members of the Tasmanian, Belgian and European parliaments; and controversies such as President Bill Clinton's environmental policies, dams on the Danube and the Amsterdam 'car-free city' referendum.

These succinct analyses are an indispensable fund of information that should give the journal a readership among nonspecialists and students. Its compre-

hensive book reviews are also an invaluable resource.

Altogether, the increasing importance of its subject and the high quality of its contents make this journal an essential requirement on the shelves of any self-respecting environmental library.

By contrast, *Greener Management International* is an odd beast. A product of the recent boom in publications on the 'greening of business', it seems to fall between the stools of a popular magazine and an academic journal.

It contains studies of particular companies' environmental strategies, articles on general topics such as environmental technologies, and policy analyses from a business perspective. But the papers are too simple and short (6–10 pages) to be of much use to management studies scholars — these issues are now well covered in specialist management journals. More general readers from within the business world will find material of interest — there is a repeated listing of green business clubs, and a belated summary of international environmental news — but the price and slim contents do not seem designed to attract them.

There is a great need for business managers to get advice on environmental good practice and to learn from the experience of others; but I doubt that this publication will prove to be the means. □

Michael Jacobs is at the Centre for the Study of Environmental Change, University

Journals also submitted for review

The following is a list of journals received that were eligible for review but which for one reason or another are not covered in the preceding pages. The list does not include journals sent in that had not published enough titles to be considered.

Applied Categorical Structures (Kluwer)
Applied Immunohistochemistry (Raven)
Applied and Preventive Psychology: Current Scientific Perspectives (Cambridge University Press)
CQ: Cambridge Quarterly of Healthcare Ethics (Cambridge University Press)
Computer Supported Cooperative Work (Kluwer)
Computational Optimization and Applications (Kluwer)
Control Engineering Practice: A Journal of the International Federation of Automatic Control (Pergamon)
Distributed and Parallel Databases (Kluwer)
Distributed Systems Engineering (Institute of Physics Publishing)

Eating Disorders Review (Wiley)
Group Decision and Negotiation (Kluwer)
Harvard Review of Psychiatry (Mosby)
Health Care Analysis: Journal of Health Philosophy and Policy (Wiley)
Human Environment (Ingvar Palmlund)
InflammoPharmacology (Kluwer)
International Journal of Uncertainty, Fuzziness and Knowledge-Based Systems (World Scientific)
Journal of Biological Systems (World Scientific)
Journal of Intelligent Information Systems (Kluwer)
Journal of Logic, Language and Information (Kluwer)
Journal of Russian Technology (International Venture)
Location Science (Pergamon)
MAGMA: Magnetic Resonance Materials — Physics, Biology, Medicine (Chapman and Hall)
Modelling and Simulation in Materials Science and Engineering (Institute of Physics Publishing)

Mucosal Immunology Update (Raven)
Multimedia Systems (Springer)
Oral Oncology: European Journal of Cancer Part B (Pergamon)
Parallel Algorithms and Applications (Gordon and Breach)
Plasma Sources: Science and Technology (Institute of Physics Publishing)
Potential Analysis (Kluwer)
Random Computational Dynamics (Dekker)
SAR and QSAR in Environmental Research (Gordon and Breach)
Set-Valued Analysis: An International Journal Devoted to the Theory of Multifunctions and Its Applications (Kluwer)
Smart Materials and Structures (Institute of Physics Publishing)
The Immunologist: Official Organ of the International Union of Immunological Societies (Hogrefe and Huber)
Transport Geography (Butterworth-Heinemann)
Transportation Research (Pergamon)
Wound Repair and Regeneration (Mosby)

PLEASE follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers who work in other fields and those for whom English is a second language, and write clearly and simply, avoiding unnecessary technical terminology. Space in the journal is limited, making competition for publication severe. Brevity is highly valued. One printed page of *Nature*, without display items, contains about 1,300 words.

Manuscripts are selected for publication according to editorial assessment of their suitability and reports from independent referees. They can be sent to London or Washington and should be addressed to the Editor. Manuscripts may be dealt with in either office, depending on the subject matter, and will where necessary be sent between offices by overnight courier. High priority cannot be given to pre-submission enquiries; in urgent cases they can be made in the form of a one-page fax. All manuscripts are acknowledged on receipt but fewer than half are sent for review. Those that are not reviewed are returned as rapidly as possible so that they may be submitted elsewhere without delay. Contributors may suggest reviewers; limited requests for the exclusion of specific reviewers are usually heeded. Manuscripts are usually sent to two or three reviewers, chosen for their expertise rather than their geographical location. Manuscripts accepted for publication are processed from the London office.

Nature requests authors to deposit sequence and crystallographic data in the databases that exist for this purpose.

Once a manuscript is accepted for publication, contributors will receive proofs in about 4 weeks. *Nature's* staff will edit manuscripts with a view to brevity and clarity, so contributors should check proofs carefully. Manuscripts are generally published 2–3 weeks after receipt of corrected proofs. *Nature* does not exact page charges. Contributors receive a reprint order form with their proofs; reprint orders are processed after the manuscript is published and payment received.

Categories of paper

Review Articles survey recent developments in a field. Most are commissioned but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance.

Progress articles review particularly topical developments for a nonspecialist readership. They do not exceed 4 pages in length. Suggestions may be made to the Reviews Coordinator in the form of a brief synopsis.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text (not including figure legends) or more than six display items and should not occupy more than five pages of *Nature*.

Articles start with a heading of 50–80 words written to advertise their content in general terms, to which editors will pay particular attention. The heading does not usually contain numbers, abbreviations or measurements. The introduction to the study is contained in the first two or three paragraphs of the article, which also briefly summarize its results and implications. Articles have fewer than 50 references and may contain a few short subheadings.

Letters to *Nature* are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should have 1,000 or fewer words of text and four or fewer display items. The first paragraph describes, in not more than 150 words and without the use of abbreviations, the background, rationale and chief conclusions of the study for the particular benefit of non-specialist readers. Letters do not have subheadings and contain fewer than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research.

News and Views articles inform nonspecialist readers about new scientific advances, sometimes in the form of a conference report. Most are commissioned but proposals can be made in advance to the News and Views Editor.

Scientific Correspondence is for discussion of topical scientific matters, including those published in *Nature*, and for miscellaneous contributions. Priority is given to letters of fewer than 500 words.

Preparation of manuscripts

All manuscripts should be typed, double-spaced, on one side of the paper only. An original and four copies are required, each accompanied by artwork. If photographs are included, five sets of originals are required; for line drawings, one set of originals and four good-quality photocopies are acceptable. Reference lists, figure legends and tables should all be on separate sheets, all of which should be double-spaced and numbered. Three copies of relevant manuscripts in press or submitted for publication elsewhere should be included with submitted manuscripts, clearly marked as such. Five copies of revised and resubmitted manuscripts, labelled with their manuscript numbers, are required, together with five copies of a letter detailing the changes made.

Titles are brief and simple. Active verbs, numerical values, abbreviations and punctuation are to be avoided. Titles should contain one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name and, when known, the manuscript number. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are to be avoided and are permitted only if the parts are closely related, either experimentally or logically. Unlettered originals of photographs should be provided. Suggestions for cover illustrations, with captions and labelled with the manuscript number, are welcome. Original artwork is returned when a manuscript cannot be published.

Protein/nucleotide sequences should ideally be in the three-letter and not the single-letter code for amino acids. One column width of *Nature* can accommodate 20 amino acids or 60 base pairs.

Colour artwork. A charge of £500 per page is made as a contribution towards the cost of reproducing colour figures. Inability to pay these costs will not prevent publication of essential colour figures if the circumstances are explained. Proofs of colour artwork may be sent to contributors under separate cover from their galley proofs.

Figure legends should not exceed 300 words and ideally should be shorter. The figure is described first, then, briefly, the method. Reference to a method published elsewhere is preferable to a full description. Methods are not described in the text.

References are numbered sequentially as they appear in the text, followed by those in tables and finally by those in figure legends. Only papers published or in the press are numbered and included in the reference list. All other forms of reference should be cited in the text as a personal communication, manuscript submitted or in preparation. Text is not included in reference lists. References are abbreviated according to the *World List of Scientific Periodicals* (Butterworths, London, 1963–65). The first and last page numbers are included; reference to books includes publisher, place and date. **Abbreviations**, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used in the text.

Acknowledgements are brief and appear after the reference list; grant and contribution numbers are not allowed.

Supplementary Information is material relevant to Articles or Letters which cannot, for lack of space, be published in full, but which is available from *Nature* on request.

Submission. Manuscripts can be sent to the Editor at 4 Little Essex Street, London WC2R 3LF, UK or at 1234 National Press Building, Washington, DC 20045, USA. A telephone and fax number should be included. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value \$5' to prevent the imposition of import duty and value-added tax.

Health-care reforms and the pharmaceutical industry

Larry Botheras

As governments look ever more critically at the costs of drugs, those working in the pharmaceutical industry must become aware of the effects on their own career prospects.

In a recent article (*Nature* 367, 764;1994) I dealt with the opportunities for career development for scientists in the pharmaceutical industry (including biotechnologists). The world is not ideal, however, and it is important to point out some of the (arguably short-term) pressures that are affecting employment prospects at present.

Factors

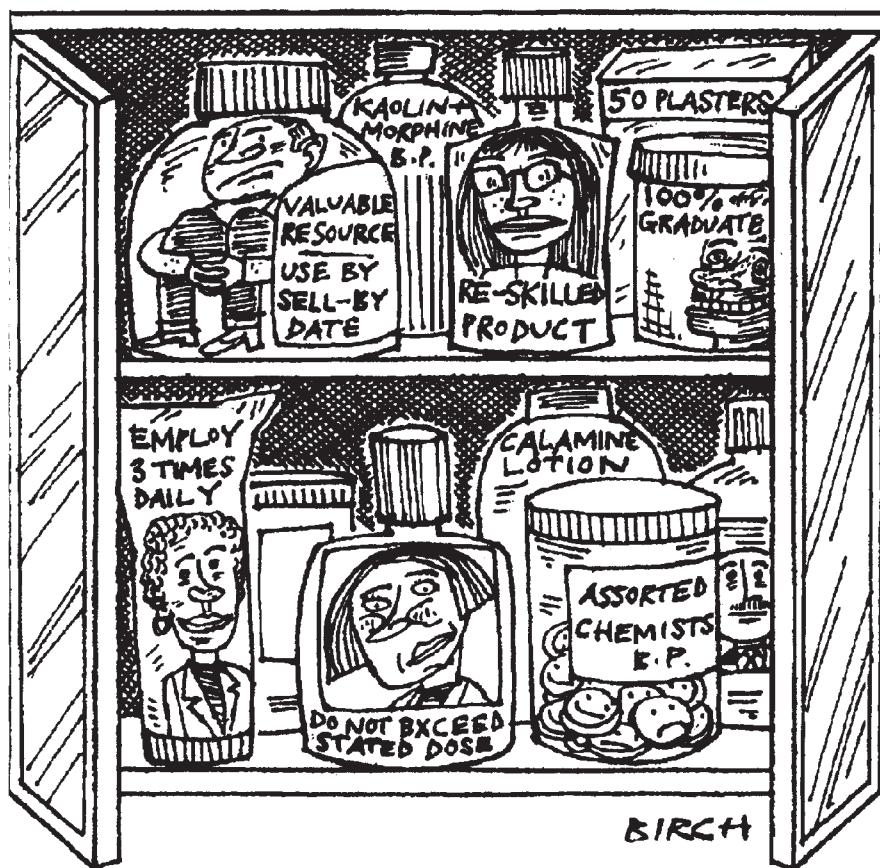
It almost goes without saying that government health-care budgets in most countries are under the strictest scrutiny, with the drug budget the principal target for cuts. In spite of the fact that spending on drugs represent a relatively small part of the overall cost of healthcare, the pharmaceutical industry is an easier opponent to take on than one of the more politically sensitive centres of cost. As a fairly secretive industry that has paid relatively little attention to expanding public knowledge of its activities, it has yet to fight back with any great effect.

There are also other factors that need to be considered. For example, the overall cost is becoming an increasingly important factor in drug registration. Already the Australian authorities require that a new product has proven cost benefits over the therapies already on the market. This approach is to be introduced in Canada as well, and the rest of the major markets are certain to follow suit. When one considers that new developments depend in the main on sophisticated high technology for their production, and are therefore expensive, the consequences of the pressure are obvious.

In addition, there is an increasing body of opinion which says that product licences should not be given to 'me too' products. Sad to say, many companies can be accused of excesses in this respect. What was the point, for example, of producing the *n*-th ACE inhibitor to join the market?

Effects

The overall effect of these changes has been to make the industry focus strongly on analysis of its business, and to develop its strategy in a way that will address its perceived strengths and weaknesses. Consequently, we are seeing some companies move out of some therapeutic areas which



they no longer see as relevant to their present R&D efforts, or which give an insufficient financial return. In tandem with this, several companies are analysing their internal 'skill set' and redefining the type of person they need to employ. This means that some companies are actually 're-skilling' — that is, making people redundant on the one hand and recruiting intensively on the other.

Employment prospects

The principal advice to give, and not only to those working in basic research, is that they should cultivate at least some appreciation of the way in which these pressures affect their own positions. For example, it is no use at all being single-minded about discovering an excellent product that deals with a condition so rare as to affect only a small number of people. The prospect of recovering the company's investment must be small in these cases,

given constraints on drug costs, and so the product has a limited future. Alternatively, a product may be directed at an established market, but have a manufacturing process so complex and a cost so high that it is impossible to imagine the product selling (probably in the face of a drug from a direct competitor which costs much less).

The future, therefore, will be one in which those who are successful have a sound understanding of why the corporation is in business and a propensity to adapt. It is to be hoped, too, that corporations will learn that they have to communicate their strategy successfully throughout the organization.

Larry Botheras is Head of Pharmaceuticals and Healthcare at Macmillan Davies, the executive search and selection consultancy based at Salisbury House, Bluecoats, Hertford, Herts, UK.